Dexmedetomidine ameliorates intracerebral hemorrhage-induced memory impairment by inhibiting apoptosis and enhancing brain-derived neurotrophic factor expression in the rat hippocampus

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Abstract. Intracerebral hemorrhage (ICH) is a severe type of stroke causing neurological dysfunction with a high mortality rate. Dexmedetomidine is an agonist for α_2-adrenoreceptors with sedative, anxiolytic, analgesic and anesthetic effects. In the present study, we investigated the effects of dexmedetomidine on short-term and spatial learning memory, as well as its effects on apoptosis following the induction of ICH in rats. A rat model of IHC was created by an injection of collagenase into the hippocampus using a stereotaxic instrument. Dexmedetomidine was administered intraperitoneally daily for 14 consecutive days, commencing 1 day after the induction of ICH. The step-down avoidance test for short-term memory and the radial 8-arm maze test for spatial learning memory were conducted. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay, immunohistochemistry for caspase-3, and western blot analysis for Bcl-2, Bax, Bid and caspase-3 expression were performed for the detection of apoptosis in the hippocampus. Western blot analysis for the brain-derived neurotrophic factor (BDNF) and tyrosine kinase B (TrkB) was also performed for the detection of cell survival in the hippocampus. The induction of ICH deteriorated short-term and spatial learning memory, increased apoptosis and suppressed BDNF and TrkB expression in the hippocampus. Treatment with dexmedetomidine ameliorated the ICH-induced impairment of short-term and spatial learning memory by suppressing apoptosis and enhancing BDNF and TrkB expression. In the normal rats, dexmedetomidine exerted no significant effects on memory function and apoptosis. The present results suggest the possibility that dexmedetomidine may be used as a therapeutic agent for the conservation of memory function in stroke patients.

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Introduction

Intracerebral hemorrhage (ICH) is a severe type of stroke causing neurological dysfunction with a high mortality rate (1). Current surgical therapies for ICH are not effective and acceptable drugs have not yet been developed for clinical trials. ICH-induced brain injury occurs through multiple mechanisms, and is also mediated in part by an apoptotic mechanism (2,3).

Apoptosis is a form of cell death that constitutes part of a common mechanism in cell replacement, tissue remodeling, and the removal of damaged cells. Apoptosis is triggered by a variety of stimuli (4); however, the inappropriate or excessive initiation of apoptosis has been implicated in several types of neurodegenerative disorders, including stroke (5). Apoptotic cell death can be assessed using terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining, which detects DNA fragmentation. The caspases, a family of 14 cysteine proteases, are essential players in apoptotic cell death both as initiators (caspase-2, -8, -9 and -10) and executioners (caspase-3, -6 and -7) (6). Cell death in the parenchyma occurs via apoptotic mechanisms during ICH, and apoptotic cell death is associated with the induction of caspase-3 in cells adjacent to the hematoma (2,7). Apart from the caspases, the Bcl-2 family proteins also play important roles in the regulation of apoptosis. The Bcl-2 family proteins are classified into anti-apoptotic proteins, including Bcl-2 and Bcl-xL, and pro-apoptotic proteins, such as Bax and Bid. The balance between pro-apoptotic and anti-apoptotic Bcl-2 family members determines the mitochondrial response to apoptotic stimuli (8).

Brain-derived neurotrophic factor (BDNF) is a small dimeric protein, and functions through its receptor, tyrosine kinase B (TrkB). BDNF modulates neuronal cell growth and survival, and BDNF has been implicated in learning and memory processes; therefore, dysfunction in BDNF is accompanied by cognitive deficits (9). BDNF enhances hippocampal-dependent memory and long-term potentiation, a form of synaptic plasticity, via TrkB (10). A high level of BDNF is concentrated in the hippocampus, and BDNF expression is selectively increased following activity-dependent learning and memory tasks (11). TrkB activation has been shown to inhibit apoptosis following subarachnoid hemorrhage (12).

Dexmedetomidine is a potent and highly selective agonist for α_2-adrenoreceptors with sedative, anxiolytic, analgesic and anesthetic effects (13,14). The neuroprotective effects of dexmedetomidine by stimulating α_2-adrenoreceptors have been reported (15-17). Dexmedetomidine has been shown to inhibit apoptotic neuronal cell death in the hippocampus by enhancing antioxidant activity following transient global cerebral ischemia-reperfusion injury in rats (18). Dexmedetomidine has also been shown to exert neuroprotective effects following subarachnoid hemorrhage-induced hippocampal injury in rabbits (19) and to reduce oxidative stress following subarachnoid hemorrhage in rats (20).

The neuroprotective effects of dexmedetomidine against several brain injuries have been suggested; however, the memory deteriorating effects of dexmedetomidine have also been indicated. Dexmedetomidine infusion results in reversible sedation, mild analgesia and memory impairment (21). Dexmedetomidine impairs long-term potentiation in the mouse hippocampus via activation of α_2-adrenoreceptors (22). van Oostrom *et al* (23) reported that the suppressive effect of dexmedetomidine on memory formation occurred at doses which reduce central nervous system activity. In a study on humans, dexmedetomidine-induced amnesia was caused by a failure of information to be encoded into long-term memory (24).

In the present study, we investigated whether dexmedetomidine ameliorates or exacerbates memory function under ICH conditions. For this purpose, a step-down avoidance test for short-term memory and a radial 8-arm maze test for spatial learning memory were conducted using rats. The anti-apoptotic effect of dexmedetomidine against ICH was also evaluated. Apoptosis in the hippocampus was detected using TUNEL assay, immunohistochemistry for caspase-3, and western blot analysis for Bcl-2, Bax, Bid and caspase-3 expression in the hippocampus. Western blot analysis for BDNF and TrkB was also performed for the detection of cell survival in the hippocampus.

Materials and methods

Experimental animals and treatments. All experiments were performed in accordance with the Animal Care Guidelines of the National Institutes of Health (NIH) and the Korean Academy of Medical Sciences. The effects of dexmedetomidine on ICH-induced brain injury in rats were evaluated. Seven-week-old Sprague-Dawley rats (210±10 g) were randomly divided into 5 groups (n=8 in each group): the sham-operated group, the ICH-induced brain injury group, the ICH-induced brain injury and 1 μg/kg dexmedetomidine-treated group, the ICH-induced brain injury and 5 μg/kg dexmedetomidine-treated group, and the ICH-induced brain injury and 10 μg/kg dexmedetomidine-treated group. In addition, the effects of dexmedetomidine on normal rats were also evaluated.

Dexmedetomidine was procured from Hospira Inc. (Rocky Mount, NC, USA). The animals in the dexmedetomidine-treated groups received the dose of dexmedetomidine in 0.5 ml saline intraperitoneally (i.p.) once a day for 14 consecutive days, commencing 1 day after the induction of ICH. The animals in the sham-operated group and the ICH-induced brain injury group received an equivalent dose of saline i.p. once a day for the same duration.

Induction of collagenase-induced ICH. To induce ICH, the rats were anesthetized with Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories, Carros, France) and placed in a stereotaxic frame. The needle of a 10-μl Hamilton syringe (Micro 701; Hamilton Co., Reno, NV, USA) was inserted through a burr hole into the right hippocampus to the following coordinates: 2.2 mm anterior and 2.2 mm lateral to the bregma, with a depth of 4.2 mm. Distilled water (1 μl) containing 0.2 U collagenase (Type 4; Sigma Chemical Co., St. Louis, MO, USA) was infused over 1 min. The needle remained in place for an additional 3 min following the infusion, and then was withdrawn slowly.

Step-down avoidance test. The latency in the step-down avoidance test was measured to evaluate short-term memory, as previously described (25). The rats were trained in the step-down avoidance test 14 days after the initiation of dexmedetomidine treatment. The rat was placed on a 7x25 cm platform that was 2.5 cm high. The platform faced a 45x25 cm grid of parallel stainless steel bars, 0.1 cm in caliber, spaced 1 cm apart. In the training session, the animal received a 0.2 mA scrambled foot shock for 2 sec immediately upon stepping down. Two hours after the training session, the latency (sec) in each group was measured. The time the rat spent on the platform before stepping down and placing all 4 paws on the grid was defined as the latency period. Latency over 180 sec was counted as 180 sec.

Radial 8-arm maze test. Spatial learning memory was tested using a radial 8-arm maze test, as previously described (26). The radial-arm maze apparatus consisted of a central octagonal plate (30 cm in diameter) and 8 radiating arms (50 cm in length and 10 cm in width). The apparatus was placed 1 m above the floor. A small receptacle filled with water (3 cm in diameter and 1 cm in depth) was located at the end of the arms. The rat was trained 3 times before the spatial learning test. During the training sessions, the rat was deprived of water for 24 h and allowed to explore the water for 5 min after finishing each session. The test was conducted on the 13th day after the initiation of dexmedetomidine treatment. The time the rat spent seeking water at the end of the arms was counted. The test was terminated when a rat found the water in all 8 arms or when >8 min elapsed. The number of correct choices made before the first wrong choice was counted, and re-entry into previously visited arms was counted as the number of wrong choices made.

Preparation of tissues. The animals were sacrificed immediately after determining the latency in the step-down avoidance test. The rats were anesthetized using Zoletil 50® (10 mg/kg, i.p.; Vibac Laboratories), transcardially perfused with 50 mM phosphate-buffered saline (PBS), and fixed with a freshly prepared solution consisting of 4% paraformaldehyde in 100 mM phosphate buffer (PB, pH 7.4). The brains were dissected and stored overnight in the same fixative solution. They were then transferred to a 30% sucrose solution for cryoprotection. For immunohistochemistry, the slices were coronal sectioned (40 μm thick) using a cryostat (Leica, Nussloch, Germany). Ten slice sections on average in the CA1 region were collected from each rat. The sections of 2.5 to 2.7 mm posterior to the bregma were used for TUNEL staining and caspase-3 immunohistochemistry.

TUNEL staining. To visualize DNA fragmentation, a marker of apoptosis, TUNEL staining was performed using an *In Situ* Cell Death Detection kit® (Roche, Mannheim, Germany), according to the manufacturer's instructions (25). The sections were post-fixed in ethanol-acetic acid (2:1) and rinsed. The sections were then incubated with proteinase K (100 μg/ml), rinsed and incubated in 3% H_2O_2, permeabilized with 0.5% Triton X-100, rinsed again, and incubated in the TUNEL reaction mixture. The sections were rinsed and visualized using a converter-POD with 0.03% 3,3'-diaminobenzidine (DAB). Mayer's hematoxylin (Dako, Glostrup, Denmark) was used as the counterstain, and the sections were mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature and dehydrated through a gradient of ethanol and covered with coverslips using Permount® (Fisher Scientific, New Jersey, NJ, USA).

Immunohistochemistry for caspase-3. To measure caspase-3 expression, caspase-3 immunohistochemistry was performed, as previously described (25). The sections from each brain were incubated overnight with mouse anti-caspase-3 antibody (1:500; Santa Cruz Biotechnology, Santa Cruz, CA, USA), and then with biotinylated mouse secondary antibody (1:200; Vector Laboratories, Burlingame, CA, USA), and were then amplified for 1 h using the Vector Elite ABC kit® (1:100; Vector Laboratories). Antibody-biotin-avidin-peroxidase complexes were visualized using 0.03% DAB, and the sections were mounted onto gelatin-coated slides. The slides were air-dried overnight at room temperature and dehydrated through a gradient of ethanol and covered with coverslips using Permount® (Fisher Scientific).

Western blot analysis. Western blot analysis was performed as previously described (26). The hippocampal tissues were dissected and collected, and were then immediately frozen at -70˚C. The right hemisphere was homogenized on ice, and lysed in lysis buffer containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM PMSF, 1 mM EGTA, 1.5 mM $MgCl_2 \cdot 6H_2O$, 1 mM sodium orthovanadate and 100 mM sodium fluoride. Protein content was measured using a Bio-Rad colorimetric protein assay kit (Hercules, CA, USA). Protein samples (30 μg) were separated on a sodium dodecyl sulfate-polyacrylamide gel and transferred onto nitrocellulose membranes.

The membranes were incubated with 5% skim milk in Tris-buffered saline containing 0.1% Tween-20 and then incubated overnight at 4˚C with the following primary antibodies: mouse anti-β-actin, anti-Bcl-2, anti-Bax, anti-caspase-3, and rabbit anti-Bid, anti-BDNF and anti-TrkB (1:1,000; Santa Cruz Biotechnology). Subsequently, the membranes were incubated for 1 h with secondary antibodies (1:2,000; Vector Laboratories), and band detection was performed using the enhanced chemiluminescence (ECL) detection kit (Santa Cruz Biotechnology).

Data analysis. For the confirmation of the expression of apoptotic proteins, the detected bands were calculated densitometrically using Molecular Analyst™, version 1.4.1 (Bio-Rad). The numbers of TUNEL-positive and caspase-3-positive cells in the hippocampal CA1 region were counted hemilaterally under a light microscope (Olympus, Tokyo, Japan), and they were expressed as the numbers of cells/mm^2 of the CA1 area. The area of the CA1 region was measured using the Image-Pro Plus image analysis system (Media Cyberbetics Inc., Silver Spring, MD, USA).

Statistical analysis was performed using one-way ANOVA followed by Duncan's post-hoc test, and the results are expressed as the means ± standard error of the mean (SEM). A P-value <0.05 was considered to indicate a statistically signficant difference.

Results

Effect of dexmedetomidine on short-term memory in the step-down avoidance test. The latency of the reactions of the rats in the step-down avoidance test is presented in Fig. 1 (left panel). The latency was decreased following the induction of ICH ($P<0.05$) and treatment with dexmedetomidine increased the latency in the rats with ICH-induced brain injury ($P<0.05$). The present results revealed that dexmedetomidine alleviated ICH-induced short-term memory impairment. In the normal rats, dexmedetomidine exerted no significant effect on latency (Fig. 2, left panel).

Effect of dexmedetomidine on spatial learning memory in the radial 8-arm maze test. The time taken to successfully perform the task, the number of correct choices made before the first wrong choice, and the number of wrong choices made before the 8 successful performances in the radial 8-arm maze test are presented in Fig. 1 (right panel). The time taken to successfully perform the task was longer, the number of correct choices made was lower, and the number of wrong choices made was higher in the rats with ICH-induced brain injury compared to the control rats ($P<0.05$). Treatment with dexmedetomidine reduced the time taken to successfully perform the task, increased the number of correct choices made, and decreased the number of wrong choices made in the rats with ICH-induced brain injury ($P<0.05$). The present results revealed that treatment with dexmedetomidine alleviated the ICH-induced spatial learning memory impairment. In the normal rats, dexmedetomidine exerted no significant effect on the time taken to successfully perform the task, the number of correct choices made, and the number of wrong choices made (Fig. 2, right panel).

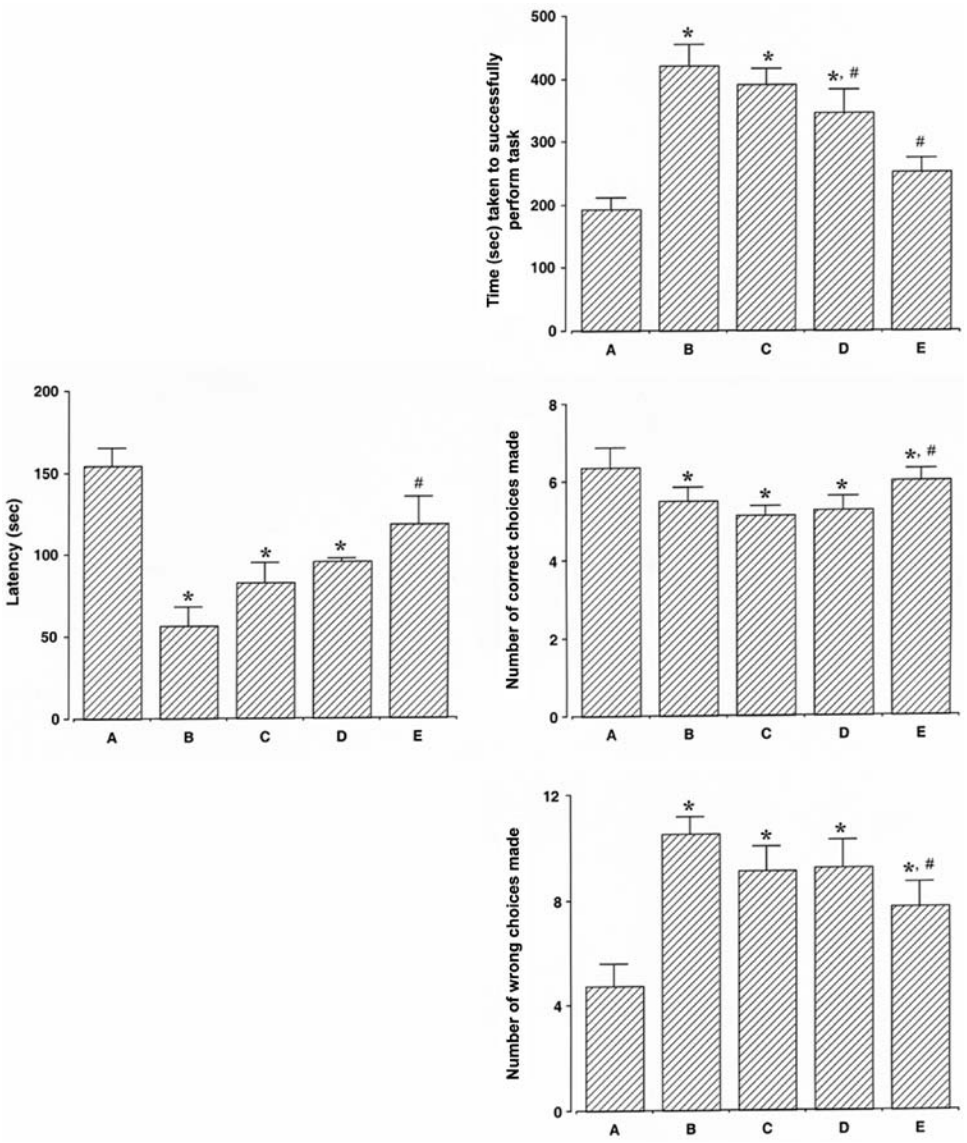

Figure 1. Effects of dexmedetomidine on short-term and spatial learning memory in rats with intracerebral hemorrhage (ICH)-induced brain injury. Left panel, step-down avoidance test; right panel, radial 8-arm maze test. (A) Sham-operated group, (B) ICH-induced brain injury group, (C) ICH-induced brain injury and 1 μg/kg dexmedetomidine-treated group, (D) ICH-induced brain injury and 5 μg/kg dexmedetomidine-treated group, (E) ICH-induced brain injury and 10 μg/kg dexmedetomidine-treated group. The results are presented as the means ± standard error of the mean (SEM). *P<0.05 compared to the sham-operated group; #P<0.05 compared to the ICH-induced brain injury group.

Effect of dexmedetomidine on the number of TUNEL-positive cells in the hippocampal CA1 region. Photomicrographs of TUNEL-positive cells in the hippocampal CA1 region are presented in Fig. 3 (upper panel). The induction of ICH increased DNA fragmentation in the CA1 region (P<0.05) and treatment with dexmedetomidine suppressed the ICH-induced DNA fragmentation (P<0.05). In the normal rats, dexmedetomidine exerted no significant effect on DNA fragmentation (Fig. 4, upper panel).

Effect of dexmedetomidine on caspase-3 expression in the CA1 region. Photomicrographs of caspase-3-positive cells in the hippocampal CA1 region are presented in Fig. 3 (lower panel). The induction of ICH increased caspase-3 expression in the CA1 region (P<0.05) and treatment with dexmedetomidine suppressed the ICH-induced caspase-3 expression (P<0.05). In the normal rats, dexmedetomidine exerted no significant effect on caspase-3 expression (Fig. 4, lower panel).

Effect of dexmedetomidine on the protein levels of Bcl-2 and Bax in the hippocampus. To determine the expression of the anti-apoptotic factor, Bcl-2, we evaluated the expression level of Bcl-2 (26-29 kDa) by western blot analysis (Fig. 5, upper left panel). The induction of ICH suppressed Bcl-2 expression in the hippocampus (P<0.05) and treatment with dexmedetomidine increased Bcl-2 expression in the rats with ICH-induced brain injury (P<0.05). In the normal rats, dexmedetomidine exerted no significant effect on Bcl-2 expression (Fig. 6, upper left panel).

To determine the expression of the pro-apoptotic factor, Bax, we evaluated the expression level of Bax (24 kDa) by western blot analysis (Fig. 5, middle left panel). The induc-

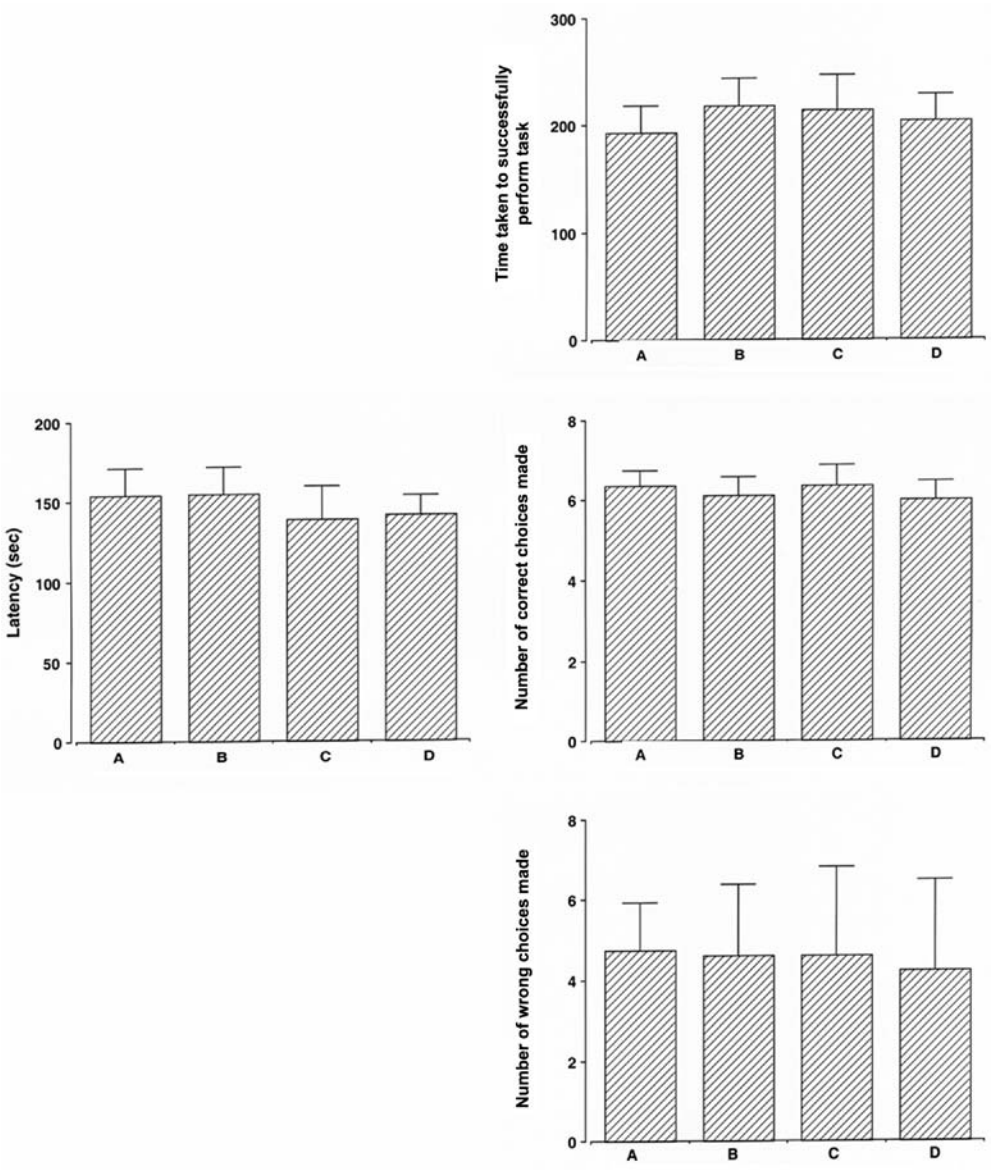

Figure 2. Effects of dexmedetomidine on short-term and spatial learning memory in normal rats. Left panel, step-down avoidance test; right panel, radial 8-arm maze test. (A) Sham-operated group, (B) sham-operated and 1 μg/kg dexmedetomidine-treated group, (C) sham-operated and 5 μg/kg dexmedetomidine-treated group, (D) sham-operated and 10 μg/kg dexmedetomidine-treated group. The results are presented as the means ± standard error of the mean (SEM).

tion of ICH increased Bax expression in the hippocampus (P<0.05) and treatment with dexmedetomidine inhibited the ICH-induced Bax expression (P<0.05). In the normal rats, dexmedetomidine exerted no significant effect on Bax expression (Fig. 6, middle left panel).

Furthermore, we analyzed the ratio of Bax to Bcl-2 (Fig. 5, lower left panel). The induction of ICH enhanced the ratio of Bax to Bcl-2 in the hippocampus (P<0.05) and treatment with dexmedetomidine suppressed the ratio of Bax to Bcl-2 in the rats with ICH-induced brain injury (P<0.05). In the normal rats, dexmedetomidine exerted no significant effect on the Bax to Bcl-2 ratio (Fig. 6, lower left panel).

Effect of dexmedetomidine on the protein expression level of Bid in the hippocampus. To determine the expression of another pro-apoptotic factor, Bid, we examined the expression level of Bid (22 kDa) by western blot analysis (Fig. 5, upper middle panel). The induction of ICH increased Bid expression in the hippocampus (P<0.05) and treatment with dexmedetomidine suppressed the ICH-induced Bid expression in the rats with ICH-induced brain injury (P<0.05). In the normal rats, dexmedetomidine exerted no significant effect on Bid expression (Fig. 6, upper middle panel).

Effect of dexmedetomidine on the protein expression level of caspase-3 in the hippocampus. To determine the expression of the apoptosis executioner, caspase-3, we evaluated the protein expression level of caspase-3 (17 kDa) by western blot analysis (Fig. 5, lower middle panel). The induction of ICH increased caspase-3 expression in the hippocampus (P<0.05) and treatment with dexmedetomidine suppressed the ICH-induced caspase 3 expression (P<0.05). In the normal rats, dexmedetomidine exerted no significant effect on caspase 3 expression (Fig. 6, lower middle panel).

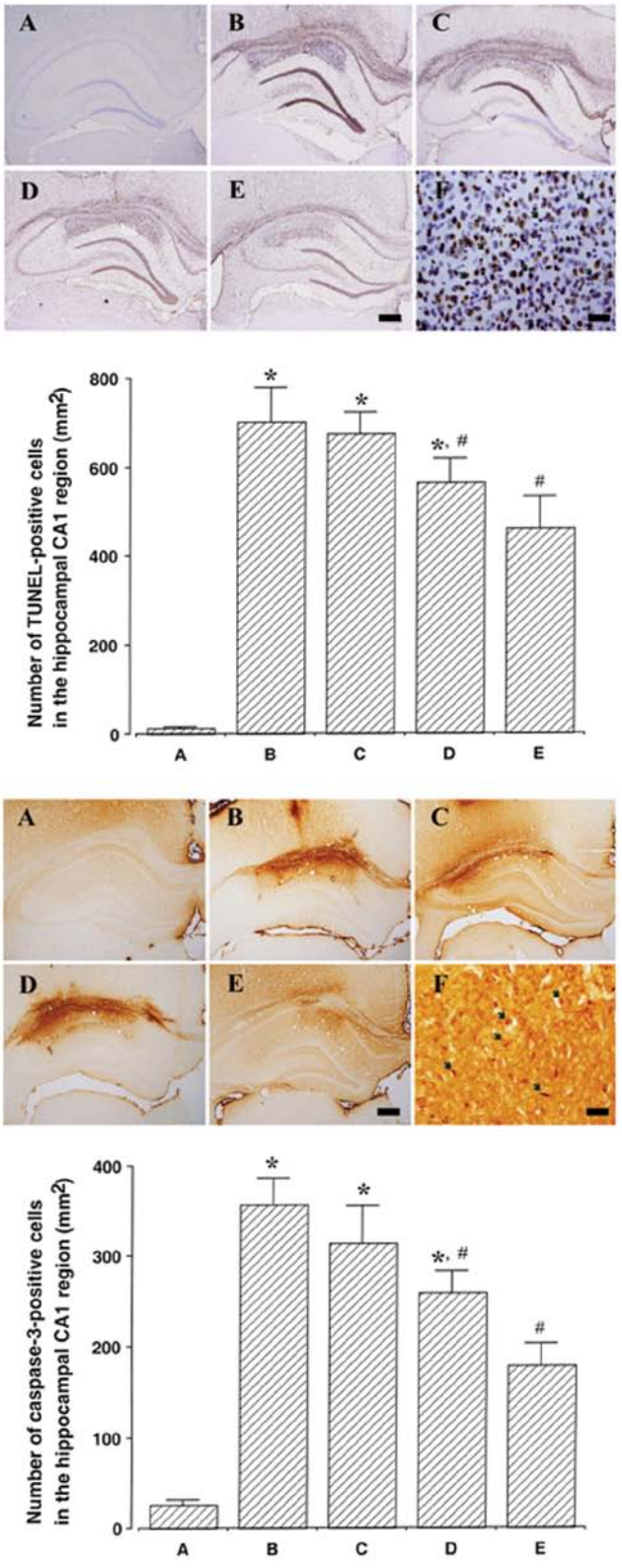

Figure 3. Effects of dexmedetomidine on DNA fragmentation and caspase-3 expression in the hippocampal CA1 region of the rats with intracerebral hemorrhage (ICH)-induced brain injury. Upper panels, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL)-positive cells; lower panels, caspase-3-positive cells. (A-E) The scale bar represents 400 μm and (F) 100 μm. (A) Sham-operated group, (B) ICH-induced brain injury group, (C) ICH-induced brain injury and 1 μg/kg dexmedetomidine-treated group, (D) ICH-induced brain injury and 5 μg/kg dexmedetomidine-treated group, (E) ICH-induced brain injury and 10 μg/kg dexmedetomidine-treated group. The results are presented as the means ± standard error of the mean (SEM). *P<0.05 compared to the sham-operated group. #P<0.05 compared to the ICH-induced brain injury group.

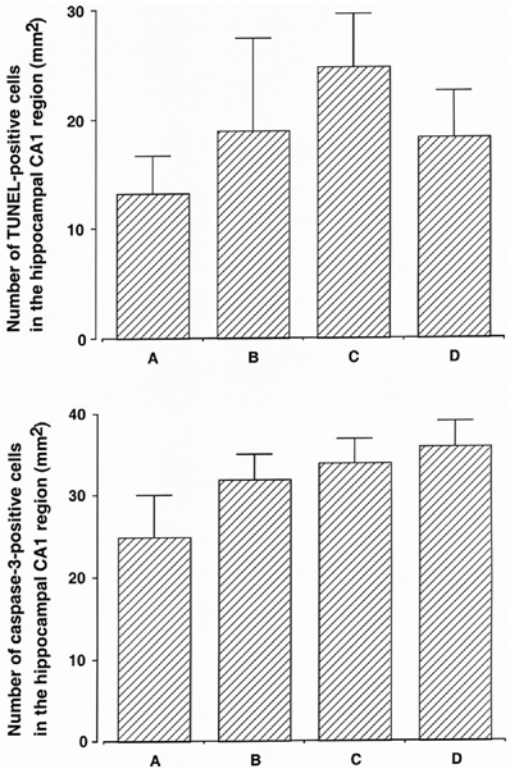

Figure 4. Effects of dexmedetomidine on DNA fragmentation and caspase-3 expression in the hippocampal CA1 region of the normal rats. (A) Sham-operated group, (B) sham-operated and 1μg/kg dexmedetomidine-treated group, (C) sham-operated and 5 μg/kg dexmedetomidine-treated group, (D) sham-operated and 10 μg/kg dexmedetomidine-treated group. The results are presented as the means ± standard error of the mean (SEM).

Effect of dexmedetomidine on the protein expression levels of BDNF and TrkB in the hippocampus. To determine the expression of BDNF and TrkB, we examined the protein levels of BDNF (15 kDa) and TrkB (95-145 kDa) by western blot analysis (Fig. 5, right panel). The induction of ICH decreased BDNF and TrkB expression in the hippocampus ($P<0.05$) and treatment with dexmedetomidine enhanced BDNF and TrkB expression in the rats with ICH-induced brain injury ($P<0.05$). In the normal rats, dexmedetomidine exerted no significant effect on BDNF and TrkB expression (Fig. 6, right panel).

Discussion

The animal model of ICH, induced by an injection of collagenase, has been used to study the mechanisms of brain injuries. The intracerebral injection of collagenase into the hippocampus induces a lesion with triggered apoptotic neuronal cell death in the hippocampus (2,3). Cognitive impairment is a common symptom following ICH, with executive and perceptual disorders being the most frequent (27). Widespread patterns of cognitive deficits have been observed in ICH patients (28).

In the present study, a step-down avoidance test showed decreased latency in the rats with ICH-induced brain injury, indicating the ICH-induced deterioration of short-term memory. Treatment with dexmedetomidine increased the latency in the rats with ICH-induced brain injury, indicating that dexmedetomidine alleviated ICH-induced short-term memory impairment (Fig. 1, left panel). A radial-arm maze

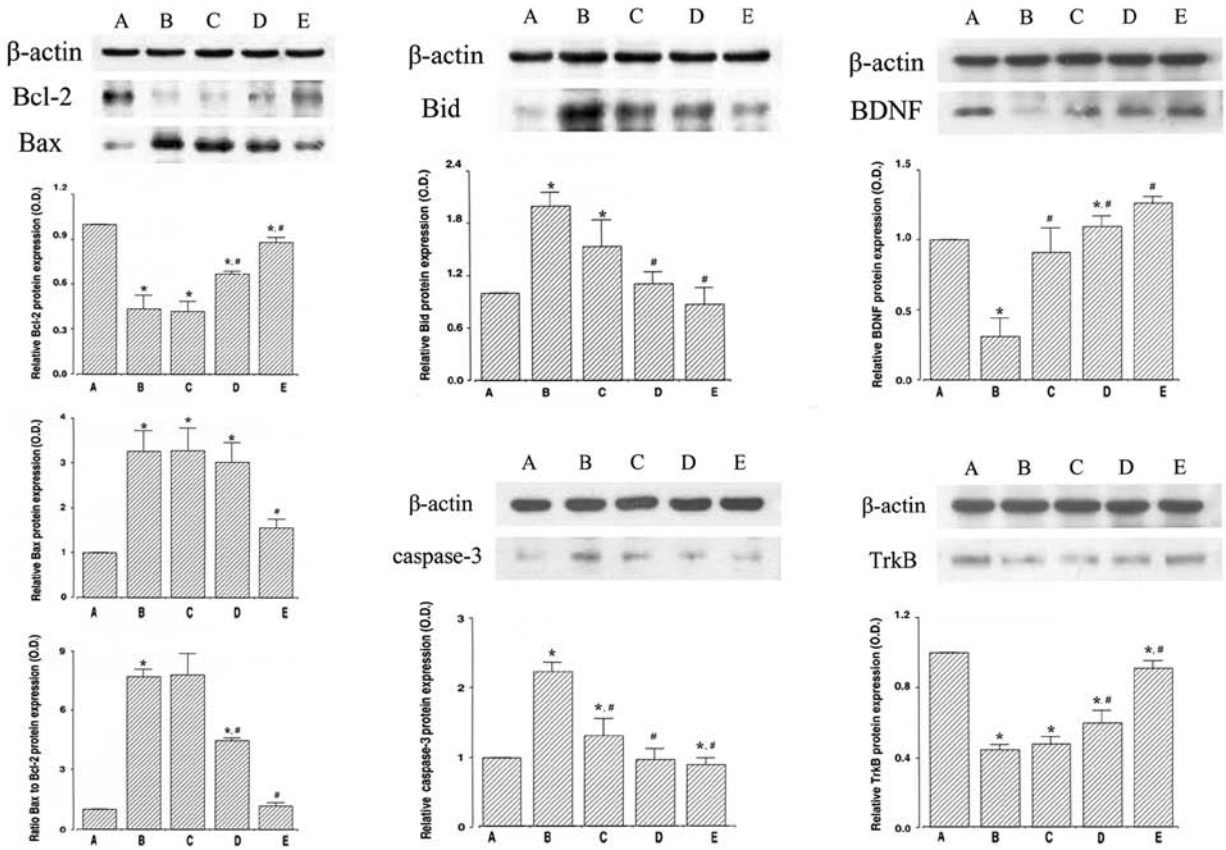

Figure 5. Effects of dexmedetomidine on the expression of apoptosis-related proteins and neurotrophic factors in the hippocampus of the rats with intracerebral hemorrhage (ICH)-induced brain injury. β-actin was used as the internal control (46 kDa). Left panel, Bcl-2 and Bax proteins; middle panel, Bid and caspase-3 proteins; right panel, brain-derived neurotrophic factor (BDNF) and tyrosine kinase B (TrkB) proteins. (A) Sham-operated group, (B) ICH-induced brain injury group, (C) ICH-induced brain injury and 1 μg/kg dexmedetomidine-treated group, (D) ICH-induced brain injury and 5 μg/kg dexmedetomidine-treated group, (E) ICH-induced brain injury and 10 μg/kg dexmedetomidine-treated group. The results are presented as the means ± standard error of the mean (SEM). *P<0.05 compared to the sham-operated group; #P<0.05 compared to the ICH-induced brain injury group.

test also showed a longer time taken to successfully perform the task, a lower number of correct choices made, and a higher number of wrong choices made in the rats with ICH-induced brain injury, indicating the ICH-induced deterioration of spatial learning memory. Treatment with dexmedetomidine shortened the time taken to successfully perform the task, increased the number of correct choices made, and decreased the number of wrong choices made in the rats with ICH-induced brain injury, demonstrating that dexmedetomidine alleviated the ICH-induced impairment of spatial learning memory (Fig. 1, right panel).

Hypoxic ischemia injury has been shown to induce short-term memory deterioration in a step-down avoidance test, as well as the impairment of spatial learning memory in a radial 8-arm maze test (29). In a previous study, maternal separation induced a decrease in latency in a step-down avoidance test, representing memory loss. By contrast, the increase in latency (to what are considered normal levels) indicated the alleviation of memory loss (30).

In this study, the numbers of TUNEL-positive and caspase-3-positive cells were increased following the induction of ICH. By contrast, treatment with dexmedetomidine decreased the numbers of TUNEL-positive and caspase-3-positive cells in the rats with ICH-induced brain injury (Fig. 3). In addition, the expression of Bid and caspase-3 increased following the induction of ICH. By contrast, treatment with dexmedetomidine suppressed the expression of Bid and caspase-3 in the rats with ICH-induced brain injury (Fig. 5, middle panel). Our results demonstrated that dexmedetomidine treatment alleviated ICH-induced apoptosis in the hippocampus.

Apoptosis appears to play a key role in neuronal cell death during stroke (31,32). The induction of ICH in rats has been shown to induce neuronal cell death, and apoptosis has been closely implicated in ICH-induced neuronal cell death (7,31). The upregulation of caspase-3 is an important hallmark of apoptosis following ischemic and hemorrhagic brain insults (3,33). An increase in the numbers of TUNEL-positive and caspase-3-positive cells in the hippocampus indicates an enhancement of apoptotic neuronal cell death in the hippocampus (3,30). The pro-apoptotic molecule, Bid, contributes to the demise of nerve cells following cerebral ischemia by the release of cytochrome *c* and activation of caspases (34). Bid expression in the hippocampus has also been shown to be upregulated following transient global cerebral ischemia in rats (35).

In our study, the expression of Bcl-2 was downregulated and that of Bax was upregulated in the hippocampus following the induction of ICH, resulting in an increase in the ratio of Bax to Bcl-2. Treatment with dexmedetomidine enhanced Bcl-2 expression and suppressed Bax expression in the rats

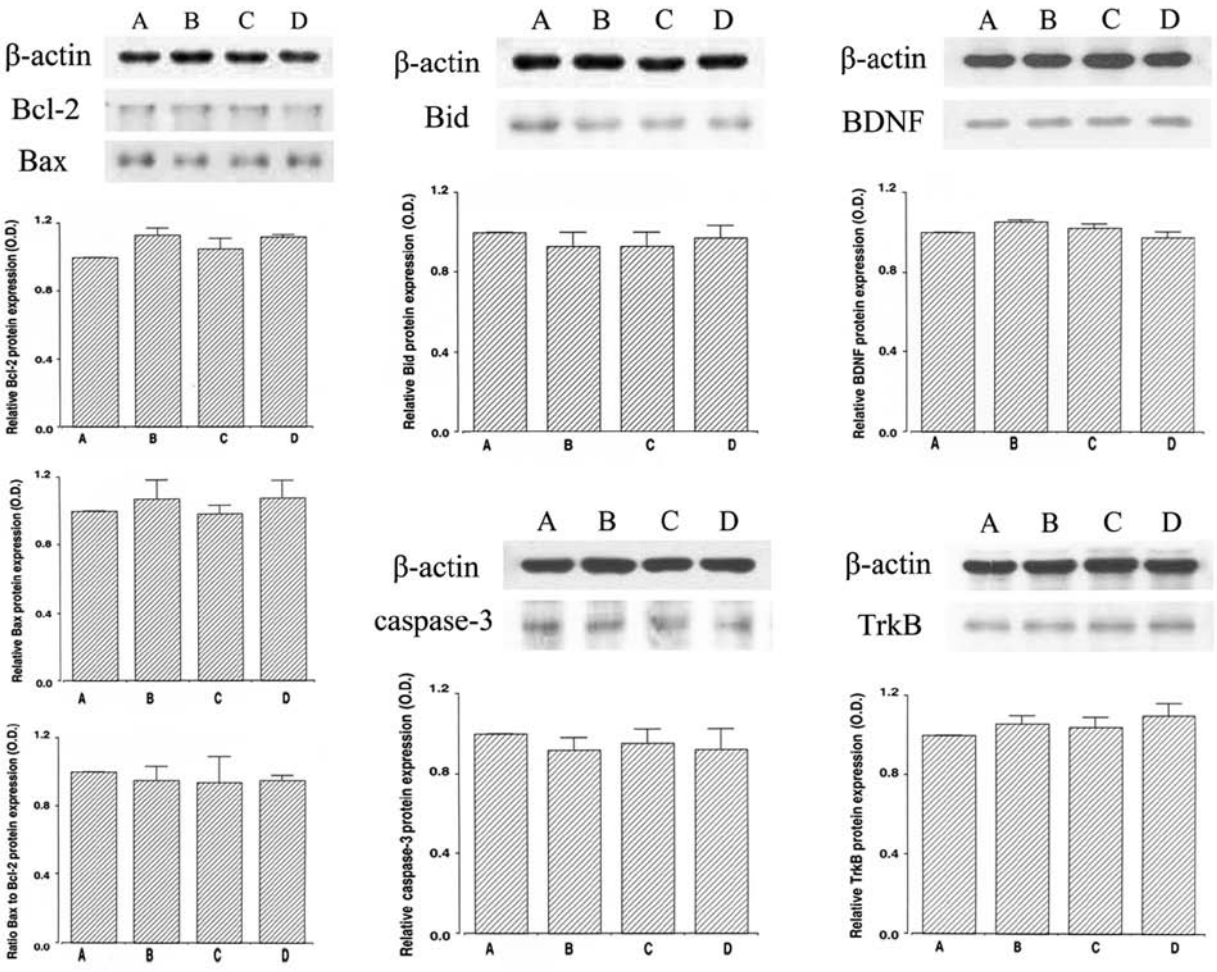

Figure 6. Effects of dexmedetomidine on the expression of apoptosis-related proteins and neurotrophic factors in the hippocampus of the normal rats. β-actin was used as the internal control (46 kDa). Left panel, Bcl-2 and Bax proteins; middle panel, Bid and caspase-3 proteins; right panel, brain-derived neurotrophic factor (BDNF) and tyrosine kinase B (TrkB) proteins. (A) Sham-operated group, (B) sham-operated and 1 μg/kg dexmedetomidine-treated group, (C) sham-operated and 5 μg/kg dexmedetomidine-treated group, (D) sham-operated and 10 μg/kg dexmedetomidine-treated group. The results are presented as the means ± standard error of the mean (SEM).

with ICH-induced brain injury, resulting in a decrease in the ratio of Bax to Bcl-2 (Fig. 5, left panel). Our results demonstrated that dexmedetomidine exerted its anti-apoptotic effects through the upregulation of Bcl-2 and the downregulation of Bax expression in the hippocampus of rats with IHC-induced brain injury.

The Bcl-2 family proteins have one or more Bcl-2 homology domains and play a crucial role in intracellular apoptotic signal transduction by regulating the permeability of the mitochondrial membrane. Bax, Bcl-xL, Bak, Bid and Bad are pro-apoptotic, and they eliminate the mitochondrial membrane potential by affecting the permeability transition pore and facilitating the release of cytochrome *c*. Conversely, Bcl-2 and Bcl-xL function to conserve the membrane potential and block the release of cytochrome *c* (36). Bcl-2 and Bcl-xL form heterodimers with the main pro-apoptotic member, Bax, and they are thus incapacitated from their protective function (37). Thus, the balance of Bax/Bcl-2 is one of the crucial factors determining whether the cells undergo apoptosis (8). In the study of Engelhard *et al* (38), dexmedetomidine upregulated Bcl-2 expression, and they suggested that the neuroprotective property of dexmedetomidine involves the modulation of the balance between pro- and anti-apoptotic proteins. Blockade of α_2-adrenoreceptors has been shown to decrease the Bax mRNA level and increase the Bcl-xL mRNA level in the cortex and hippocampus, indicating anti-apoptotic effects (39). Dexmedetomidine has also been shown to inhibit isoflurane-induced cortical apoptosis, and this protective effect of dexmedetomidine was achieved by the reversal of the isoflurane-induced decrease in Bcl-2 expression (40).

In this study, the expression of BDNF and TrkB was suppressed in the hippocampus following the inductionv of ICH. By contrast, dexmedetomidine treatment increased BDNF and TrkB expression in the rats with ICH-induced brain injury (Fig. 5, right panel). The results from the present study indicate that the reduced BDNF and TrkB expression in the hippocampus is involved in the short-term and spatial learning memory impairment induced by ICH. The increase in BDNF and TrkB expression following treatment with dexmedetomidine ameliorated the ICH-induced memory deficits.

BDNF is involved in neuronal survival and differentiation, and plays an important role in the learning process due

to its involvement in long-term potentiation in the hippocampus (9,10,41). Plasminogen activator (tPA), by activating the extracellular protease plasmin, converts the precursor proBDNF to the mature BDNF and mature BDNF is a key protein for long-term potentiation (42). BDNF expression in the hippocampus has been shown to be suppressed following traumatic brain injury and hemorrhage hypotension, suggesting that BDNF exerts neuroprotective effects (30,43). The enhanced BDNF expression in the hippocampus improves both short-term and long-term memory, and contributes to neuronal survival and differentiation (44,45). The age-induced loss of short-term and spatial working memory is accompanied with the suppression of BDNF expression in the hippocampus, while the increased BDNF expression contributes to memory enhancement (26). BDNF with fibrin-binding domain significantly reduce the hematoma volume, alleviate tissue loss, promote neuronal cell regeneration and improve behavioral performance in rats with ICH-induced brain injury (46).

As mentioned above, the anti-apoptotic and neuroprotective effects of dexmedetomidine against various brain insults have been well documented. However, sedative-hypnotic drugs are known to impair memory function; however, the details regarding the nature of these effects are unknown. The memory-deteriorating effects of dexmedetomidine have also been reported (22-24).

In the present study, we focused on the memory-enhancing effects of dexmedetomidine under ICH conditions. The present results showed that dexmedetomidine ameliorated ICH-induced memory impairment. Dexmedetomidine also exerted anti-apoptotic effects and increased BDNF expression in the hippocampus of rats with ICH-induced brain injury. In the normal rats, dexmedetomidine exerted no significant effects on apoptosis and memory function, indicating that dexmedetomidine exerts no detrimental effects on normal rats (Figs. 2, 4 and 6). Based on the present results, dexmedetomidine may be used as a therapeutic agent for the conservation of memory function in stroke patients.

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