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Dexmedetomidine protects neurons from kainic acid-induced excitotoxicity by activating BDNF signaling

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ABSTRACT

Glutamatergic excitotoxicity is crucial in the pathogenesis of epileptic seizures. Dexmedetomidine, a potent and highly selective α2 adrenoceptor agonist, inhibits glutamate release from nerve terminals in rat cerebrocortical nerve terminals. However, the ability of dexmedetomidine to affect glutamate-induced brain injury is still unknown. Therefore, the present study evaluated the protective effect of dexmedetomidine against brain damage by using a kainic acid (KA) rat model, a frequently used model for temporal lobe epilepsy. Rats were treated with dexmedetomidine (1 or 5 μg/kg, intraperitoneally) 30 min before the KA (15 mg/kg) intraperitoneal injection. KA-induced seizure score and elevations of glutamate release in rat hippocampi were inhibited by pretreatment with dexmedetomidine. Histopathological and TUNEL staining analyzes showed that dexmedetomidine attenuated KA-induced neuronal death in the hippocampus. Dexmedetomidine ameliorated KA-induced apoptosis, and this neuroprotective effect was accompanied by inhibited the KA-induced caspase-3 expression as well as MAPKs phosphorylation, and reversed Bcl-2 down-expression, coupled with increased Nrf2, BDNF and TrkB expression in KA-treated rats. The results suggest that dexmedetomidine protected rat brains from KA-induced excitotoxic damage by reducing glutamate levels, suppressing caspase-3 activation and MAPKs phosphorylation, and enhancing Bcl-2, Nrf2, BDNF and TrkB expression in the hippocampus. Therefore, dexmedetomidine may be beneficial for preventing or treating brain disorders associated with excitotoxic neuronal damage. In conclusion, these data suggest that dexmedetomidine has the therapeutic potential for treating epilepsy.

1. Introduction

Epilepsy is a neurological disorder that is characterized by recurrent spontaneous seizures. It is one of the most common brain disorders and affects approximately 50 million people worldwide. Although the exact cause of seizures remains unclear, a weight of evidence suggests that excessive release of glutamate is involved in the pathogenesis of epileptic seizures. For example, the intracerebral administration of glutamate receptor agonists has been demonstrated to induce seizures in animals (Chapman, 1998; Loscher, 1998). However, antagonists of glutamate receptors are powerful anticonvulsants that reduce brain damage in many animal models of epilepsy (Chapman et al., 2000; Clifford et al., 1990). Furthermore, studies have reported elevated glutamate levels in both experimental epilepsy models and human epilepsy patients (Chapman et al., 1996; During and Spencer, 1993; Wilson et al., 1996). Some antiepileptic drugs have been proven to inhibit the release of glutamate in human and rat brain tissues (Kammerer et al., 2011; Sitges et al., 2007a, 2007b). Therefore, reducing the release of excitatory neurotransmitter glutamate and inhibiting glutamate receptor function may be promising targets for antiepileptic drugs.

Dexmedetomidine is a potent and highly selective α2 adrenoceptor

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agonist with sedative, analgesic, and anesthetic properties. The neuroprotective effects of dexmedetomidine have been reported in various brain-injury models. An increasing amount of evidence indicates that dexmedetomidine protects against ischemia-induced neuronal injuries (Rajakumaraswamy et al., 2006; Sato et al., 2010), attenuates glutamate-mediated excitotoxicity in cortical neuron cultures (Ma et al., 2004) in animals (Paris et al., 2006), and reduces anesthetic-induced neuronal death (Sanders et al., 2010) and cognitive impairment (Sanders et al., 2009). Our study revealed that dexmedetomidine reduces the release of glutamate from nerve terminals (Chiu et al., 2011) suggesting the potential of dexmedetomidine to attenuate glutamate excitotoxicity. However, the mechanisms underlying the neuroprotective function of dexmedetomidine are not yet fully understood. In this study, we used an animal model injected with kainic acid (KA) to evaluate the neuroprotective effect of dexmedetomidine on epileptic rats. KA is an analog of excitotoxic glutamate, and its systemic administration in rodents causes recurrent seizures and subsequent degeneration of selective populations of neurons in the brain, particularly in the CA3 area of the hippocampus (Jarrard, 2002). Moreover, KA-induced hippocampal neuronal death is believed to be mediated by excessive release of glutamate (Friedman et al., 1994). Therefore, KA is commonly employed to investigate the mechanisms of neurodegeneration induced by excitotoxicity in order to propose pharmacological agents with potential neuroprotective effects (Wang et al., 2005).

2. Materials and methods

2.1. Animals

A total of 100 adult male Sprague-Dawley rats weighing 240–250 g were used for this study. The rats were purchased from BioLASCO (Taipei, Taiwan). All procedures in experimental animals were carried out according to the National Institutes of Health guide for the care and use of laboratory animals, and under the supervision and with the approval of Far Eastern Memorial Hospital Animal Care and Utilization Committee (106-02-11). All possible efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Kainic acid and evaluation of seizure activity

Dexmedetomidine, KA was bought from Sigma Aldrich (St. Louis, MO, USA). Animal were randomly divided into the following groups: saline + saline (control) group; KA 15 mg/kg + saline group; KA + dexmedetomidine 1 μg/kg group and KA + dexmedetomidine 5 μg/kg group. Seizures were induced by intraperitoneal (i.p.) administration of rats with KA (15 mg/kg) in saline. Adult male SD rats were treated with saline or dexmedetomidine (i.p.) 30 min before KA injection. Experimental animals were monitored for 4 h after the KA injection to determine the severity of seizures. KA induced behavioral seizure activity is rated by a scale devised by Racine. Wet dog shakes (WDS), facial clonus and staring are given a seizure score of stage 1; head nodding is stage 2; forelimb clonus is stage 3; forelimb clonus with rearing is stage 4; rearing jumping, falling and status epilepticus is stage 5. Three days after the KA treatment, the effect of dexmedetomidine on rat hippocampal neurons was analyzed using Fluro-Jade B sating and neutral red staining.

2.3. Glutamate assay

Glutamate content of rat hippocampus tissues was measured as previous described (Morishima et al., 2005). Rats were killed through decapitation three days after KA injection. The hippocampus was collected and homogenized in HEPES buffer. The supernatant was filtered in an Ultra free-MC microcentrifuge filters (Millipore, Darmstadt, German). After centrifugation, 10 μl of the filtrate was then injected into a high-performance liquid chromatography (HPLC) analysis system with an electrochemical detection (HTEC-500, Eicom, Kyoto, Japan). The HPLC conditions were as follows: separation column Eicom GU-GEL (4.6 × 150 mm); Enzyme Column: Eicom E-ENZYMPAK (3 × 4 mm); pre-column, EICOMPAK CH-GEL in PC-04 (4 × 5 mm, Eicom USA, San Diego, CA, USA); mobile phase: 60 mM NH_4Cl–NH4OH containing hexadecyltrimethylammonium bromide (HDTA) including 0.05 mg/L EDTA•2Na; flow rate 370 μL/min; System Temperature 33 °C and a platinum electrode set at 450 mV against an Ag/AgCl reference electrode.

2.4. Neutral red staining

After behavioral assessment, rats were sacrificed 3 days after KA injection with an overdose of anesthetics (chloral hydrate, 650 mg/kg, i.p.). The animals were then perfused transcardially with saline followed by cold 4% paraformaldehyde in 0.1 M PBS. The brains were removed immediately and post-fixed in the same fixative overnight at 4 °C, and then cryoprotected in 30% sucrose for 24–48 h.

For neutral red staining, the frozen brains were sectioned coronally at a thickness of 30 μm in a cryostat at −20 °C. The sections were mounted on silane-coated glass slides, air dried and then stained with neutral red solution. Neutral red was obtained from Sigma Aldrich (St. Louis, MO, USA).

2.5. Fluoro-Jade B staining

Fluoro-Jade B (Chemicon, Millipore Ltd, California, USA), a high affinity fluorescent marker for all degenerating neurons, was used to quantify cell loss. Staining for Fluoro-Jade B was performed as described previously (Schmued and Hopkins, 2000). The sections (10 μm) were mounted on silane-coated slides and dried at room temperature followed by a solution containing 1% sodium hydroxide in 80% ethanol for 5 min. After the slides were immersed in 70% ethanol for 2 min and in distilled water for 2 min, the sections were oxidized in 0.06% potassium permanganate for 15 min, washed with water, and then immersed in 0.001% Fluoro-Jade B solution for 30 min in the dark. The slides were then washed in distilled water, air dried, cleared, and coverslipped. According to previous studies (Friedman et al., 1994; Park et al., 2008), the hippocampus CA3 is the most vulnerable area to excitotoxic lesions caused by KA. Therefore, the CA3 region was visualized under 100X magnification using an upright fluorescence microscope (Zeiss Axioskop 40, Goettingen, Germany) and digitized photomicrographs used for analysis were captured using a digital camera (Nikon D80, Tokyo, Japan) between bregma −2.30 mm and −3.60 mm according to the rat brain atlas of Paxino and Watson (Paxinos and Watson, 1998). To compare neuronal death among the experimental groups, the number of Fluoro-Jade B-positive cells was measured in a 255 × 255 m area of the hippocampal CA3 in 6–8 randomly chosen sections from each animal and averaged for each animals using a computer-assisted image analysis system (Image J; NIH Image, National Institutes of Health, Bethesda, MD, USA) by an examiner blind to experimental conditions. Results were expressed as mean ± SEM of labeled cells per 0.1 mm^2.

2.6. TUNEL staining

To visualize the apoptotic cells, TUNEL (Terminal deoxynucleotidyl transferase dUTP nick end labeling) staining was performed using an In Situ Cell Death Detection Kit, TMR red according to the manufacturer's instruction. Briefly, the brain sections were treated with freshly prepared permeabilisation solution (0.1% (v/v) Triton X-100 in 0.1% sodium citrate) and then washed in cold PBS and incubated with TUNEL stain mixture for 60 min at 37 °C in a humidified atmosphere. The apoptosis of neuronal cells was observed by fluorescence microscopy. In Situ Cell Death Detection Kit, TMR red Kit was purchased from Roche (Mannheim, Germany).

2.7. Western blotting

At 24 h or 3 days after KA injection, the rats were killed by decapitation and hippocampus rapidly dissected. Different time points were chosen on the basis of previous study (Chiu et al., 2015; Jeon et al., 2000; Lin et al., 2013). Western blot was conducted as described previously (Lin et al., 2013). The membranes were incubated with primary antibodies (rabbit monoclonal anti-cleaved caspase-3, 1:1000; rabbit polyclonal anti-Bcl-2, 1:1000; rabbit monoclonal anti-BDNF, 1:1000; rabbit polyclonal anti-TrkB, 1:1000; rabbit monoclonal anti-phospho-p44/42 (ERK 1/2) MAPK, 1:1000; rabbit monoclonal anti-phospho-p38 MAPK, 1:1000; rabbit monoclonal anti-phospho-SAPK/JNK, 1:1000; rabbit monoclonal anti-p44/42 MAPK (ERK 1/2), 1:1000; rabbit monoclonal anti-p38 MAPK, 1:1000; rabbit monoclonal anti-SAPK/JNK, 1:1000 and rabbit polyclonal anti-Nrf2, 1:1000.) overnight at 4 °C. After three washes in Tris-buffered saline, the membrane was treated with the secondary horse radish peroxidase-conjugated antibody (1:3000) for 1 h. The membranes were then washed three times and visualized using the enhanced chemiluminescence system (Amersham, Buckinghamshire, UK). The level of expression or phosphorylation was assessed by band density, which was quantified by densitometry. Densitometric quantification of bands was analyzed using Multi Gauge software (FUJIFILM, Tokyo, Japan).

2.8. Statistical analysis

Data were expressed as mean ± SEM. The data reported were analyzed by using one-way ANOVA accompanied by post-hoc LSD comparison tests for multiple comparisons. The analysis was completed using SPSS software (17.0; SPSS Inc., Chicago, IL). $P < 0.05$ was considered to represent a significant difference.

3. Results

3.1. Dexmedetomidine pretreatment reduces KA-induced neuronal cell death in the CA3 area of the hippocampus

Dexmedetomidine was administered (i.p.) in 1 and 5 μg/kg doses 30 min prior to KA administration for the purpose of investigating its neuroprotective effects. The neuronal death and apoptosis in the CA3 subfield at three days after KA treatment (15 mg/kg, i.p.) was examined using neutral red, Fluoro-Jade B, and TUNEL staining. Neutral red staining revealed significant neuronal loss in the KA-treated group compared with that in the saline-treated group (control) (Fig. 1A1, A2). Dexmedetomidine injection substantially reduced KA-induced neuronal death in CA3 (Fig. 1A3, A4). A similar protective effect against neuronal death from dexmedetomidine was observed with Fluoro-Jade B staining (Fig. 1B1–B4). As illustrated in Fig. 1B1, no staining occurred in the control group. KA treatment caused a significant increase in the number of Fluoro-Jade B-positive neurons in the CA3 region of the hippocampus ($P < 0.001$; Fig. 1B2). In rats pretreated with dexmedetomidine (1 or 5 μg/kg), the number of Fluoro-Jade B-positive neurons in CA3 was significantly lower than in the KA-treated group ($F(2, 36) = 69.706$, $P < 0.0001$; Fig. 1B3, B4). A similar result was obtained using TUNEL staining, as shown in Fig. 3C1–C4, KA significantly increased the number of TUNEL-positive cells in the CA3 region of the hippocampus (Fig. 1C2), while dexmedetomidine blocked the response (Fig. 1 C3, C4) [$F(2, 36) = 26.645$, $P < 0.001$].

3.2. Dexmedetomidine prevents KA-induced apoptosis-related proteinexpressionin the hippocampus

To discern the potential mechanisms underlying the neuroprotective role of dexmedetomidine, we evaluated caspase-3 and B-cell lymphoma 2 (Bcl-2) protein expressions through immunofluorescence staining (Fig. 2A, C) and Western blot (Fig. 2B, D). Caspase-3 is a prominent executioner of apoptosis, and its activation requires the proteolytic processing of its inactive zymogen into P17 and P12 fragments. We compared the levels of activated caspase-3 (cleavage) in this study using an antibody specific to p17. As expected, cleaved caspase-3 was not detected in the control hippocampus, but KA injection markedly increased the number of activated caspase-3-positive neurons (Fig. 2A1, A2). Dexmedetomidine pretreatment significantly decreased the number of KA-induced caspase-3-positive cells in the hippocampal CA3 region (Fig. 2A3, A4). Additionally, dexmedetomidine hindered KA-induced increases in active caspase-3 protein expression by Western blot (Fig. 2B). A significant increase in active caspase-3 protein in the hippocampus was observed 3 days after KA administration ($P < 0.001$). Dexmedetomidine administration 30 min prior to KA treatment reduced the active caspase-3 protein expression induced by KA [$F(3, 20) = 398.44$, $P < 0.0001$; Fig. 2B]. By contrast, KA treatment significantly decreased the number of Bcl-2-positive neurons (Fig. 2C1, C2) and the protein expression levels of Bcl-2 (Fig. 2D). Treatment with dexmedetomidine significantly reversed KA-induced changes in the number of Bcl-2-positive neurons (Fig. 2C3, C4) and the reduction in Bcl-2 protein levels (Fig. 2D).

3.3. Dexmedetomidine promotes expression of the neurotrophic factors BDNF and its receptor, TrkB, in rat hippocampus

KA has been demonstrated to elevate the protein levels of brain-derived neurotrophic factor (BDNF) and its receptor, TrkB, in the hippocampus (Goutan et al., 1998). To determine whether BDNF and the TrkB receptor were involved in the neuroprotective activity of dexmedetomidine, BDNF and TrkB protein levels were determined through Western blot analysis (Fig. 3). Twenty-four hours after KA administration, immunoblotting analysis showed that KA increased BDNF [$F(3, 8) = 362.94$, $P < 0.0001$; Fig. 3A] and TrkB [$F(3, 8) = 362.94$, $P < 0.0001$; Fig. 3B] expression in the hippocampuswhen compared to saline-treated group (control). Pre-treatment with dexmedetomidine potentiated this increase when compared to KA treatment alone.

3.4. Dexmedetomidine decreases KA-induced elevation of glutamate release and attenuates KA-induced seizures

Within 10–30 min after KA was administered, all animals presented withseizure behavior that persisted for 3–4 h. The seizure latency and score were 14.8 ± 23 min and 4.4 ± 0.4, respectively (Fig. 4 A, B). Administration of dexmedetomidine (1 and 5 μg/kg, i.p.) 30 min prior to KA injection increased the seizure latency [$F(2, 22) = 9.937$, $P = 0.001$; Fig. 4A] and decreased the seizure score [$F(2, 22) = 37.851$, $P = 0.0001$; Fig. 4B].

Three days after KA treatment, extracellular glutamate levels were measured using high-performance liquid chromatography (HPLC; Fig. 4C). KA administration caused a significant increase in hippocampus glutamate levels compared with the control ($P < 0.002$). In comparison with KA treatment alone, pretreatment with dexmedetomidine significantly reduced glutamate levels ($P < 0.002$). A statistical analysis revealed significant differences in the hippocampal glutamate levels among the four groups [$F(3, 20) = 45.692$, $P < 0.0001$; Fig. 4C].

3.5. Dexmedetomidineregulates KA-induced mitogen-activated protein kinase phosphorylation and Nrf2 expression in the hippocampus

KA has been shown to activate mitogen-activated protein kinases (MAPKs), including p44/42 extracellular-signal-regulated protein kinase 1/2 (ERK 1/2), p38, and c-Jun N-terminal kinase (JNK), in the hippocampus (Jeon et al., 2000). The effect of dexmedetomidine on the phosphorylation of these kinases was determined by Western blot (Fig. 5). In Fig. 5A, a significant increase in ERK1/2 phosphorylation was observed at 3 h after KA administration, and this effect was reduced

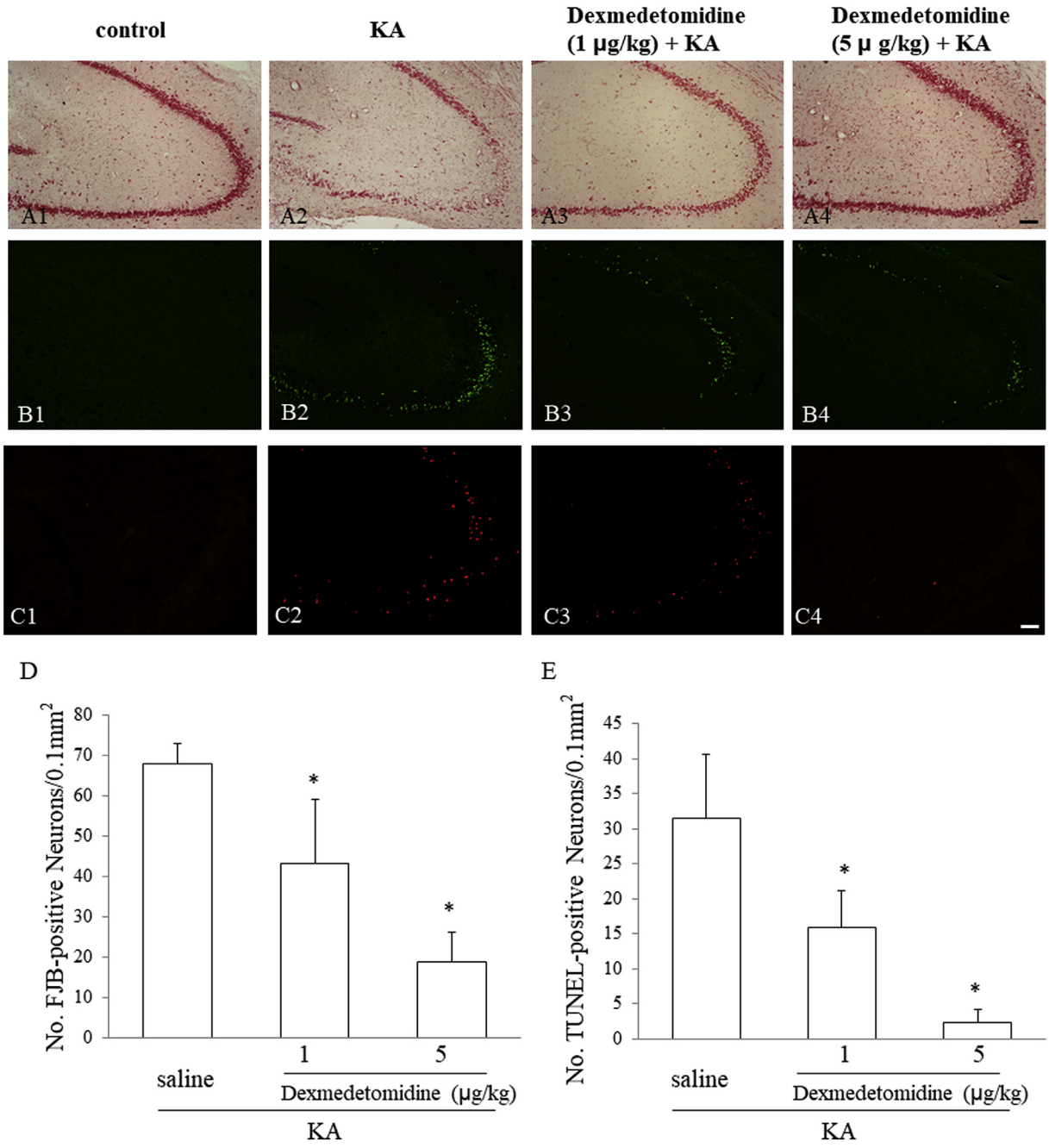

Fig. 1. Dexmedetomidine attenuates KA-induced neuronal death in the hippocampal CA3 region.

by dexmedetomidine. [F (3, 12) = 113.2, $P < 0.0001$]. In contrast to the long duration of ERK 1/2 activation, increases of JNK and p38 phosphorylation in the hippocampus were detected at 1 h after KA treatment ($P < 0.0001$). Dexmedetomidine pretreatment also blocked the KA-induced phosphorylation of p38 [F (3, 12) = 399.2, $P < 0.0001$; Fig. 5B] and JNK [F (3, 12) = 839.7, $P < 0.0001$; Fig. 5C].

In addition, to explore the role of nuclear factor-erythroid 2-related factor 2 (Nrf2) in the neuroprotective effect of dexmedetomidine, we detected the protein expression of Nrf2 in the hippocampus. Fig. 5D shows that Nrf2 level was higher in the KA group than in the saline-treated group (control) at 24 h after KA administration. Compared to KA group, dexmedetomidine pretreatment enhanced Nrf2 expression in the hippocampus [F (3, 12) = 303.9, $P < 0.0001$; Fig. 5D].

4. Discussion

Excessive release of glutamate is a major factor underlying the pathophysiology of both seizures and epilepsy. Therefore, regulating glutamate release may be acritical mechanism for antiepileptic drugs. Dexmedetomidine is a highly selective α2 adrenergic receptor agonist and has been widely used for sedation in intensive care units and operating rooms. Studies have reported the neuroprotective effects of dexmedetomidine in various models of neurotoxicity (Rajakumaraswamy et al., 2006; Sato et al., 2010), but its exact mechanisms have yet to be fully verified. In another study, we determined that dexmedetomidine depresses glutamate release from nerve terminals (Chiu et al., 2011), and therefore we hypothesized that the neuroprotective effects of dexmedetomidine may be a result of its ability to inhibit glutamate release. To confirm this hypothesis, we selected a rat

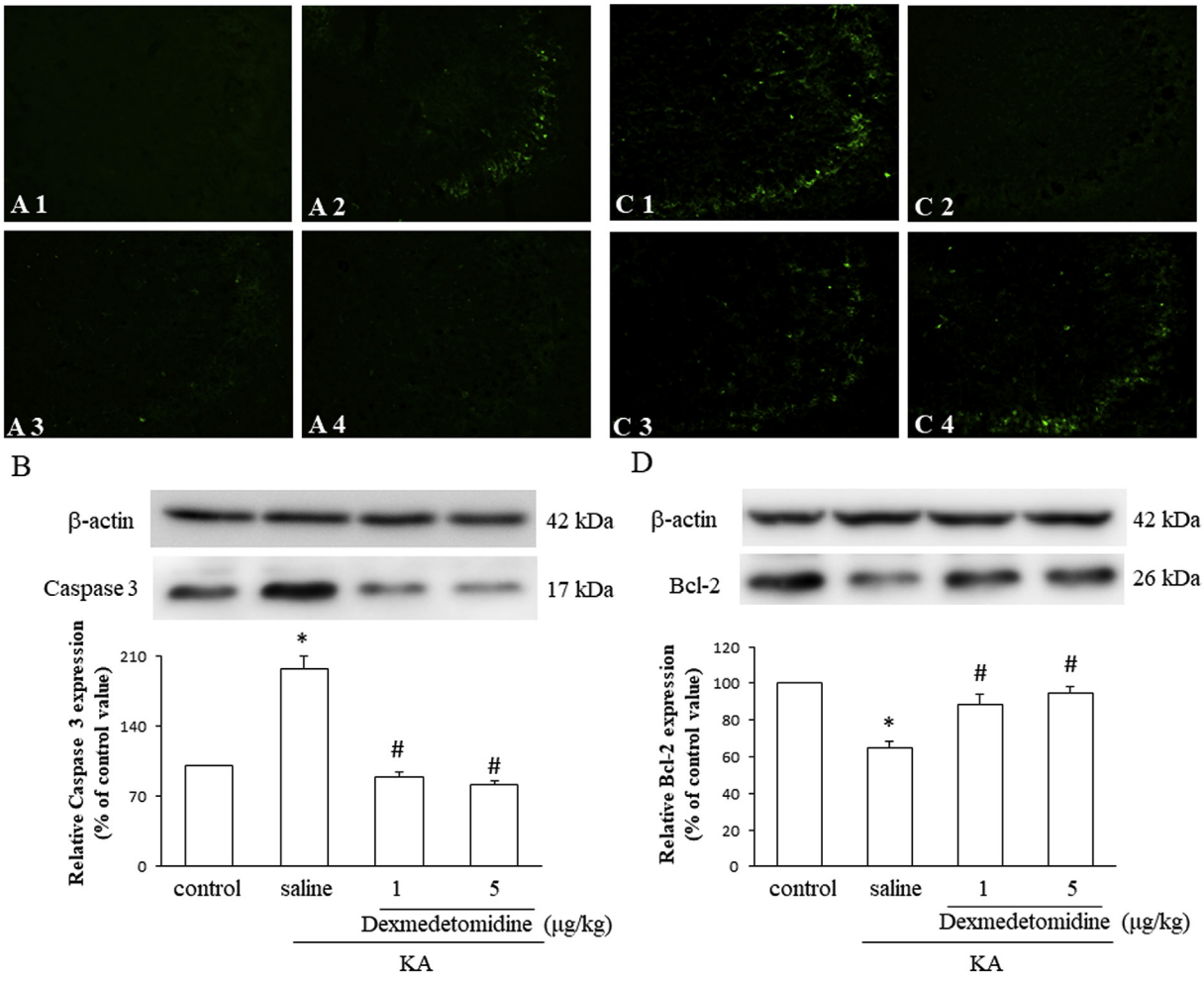

Fig. 2. Dexmedetomidine decreases the expression of apoptosis-related proteins induced by KA in the hippocampus.

model that had been treated with KA, a glutamate analog with an excitotoxic effect. This animal model was selected because KA induces recurrent seizures and neuronal death in selected brain regions of rats in a fashion that mimics the effects of human temporal-lobe epilepsy (Ben-Ari and Cossart, 2000). Moreover, these alterations induced by KA are associated with excessive release of glutamate. In the present study, the systemic administration of KA (15 mg/kg, i.p.) in rats induced epileptic seizures. The results are consistent with the observations of other experiments that applied the same dose of KA (Chiu et al., 2018; Friedman et al., 1994). Dexmedetomidine (1 and 5 μg/kg, i.p.) significantly increased the latency of seizure onset and reduced the seizure score following KA administration. This is consistent with other studies,

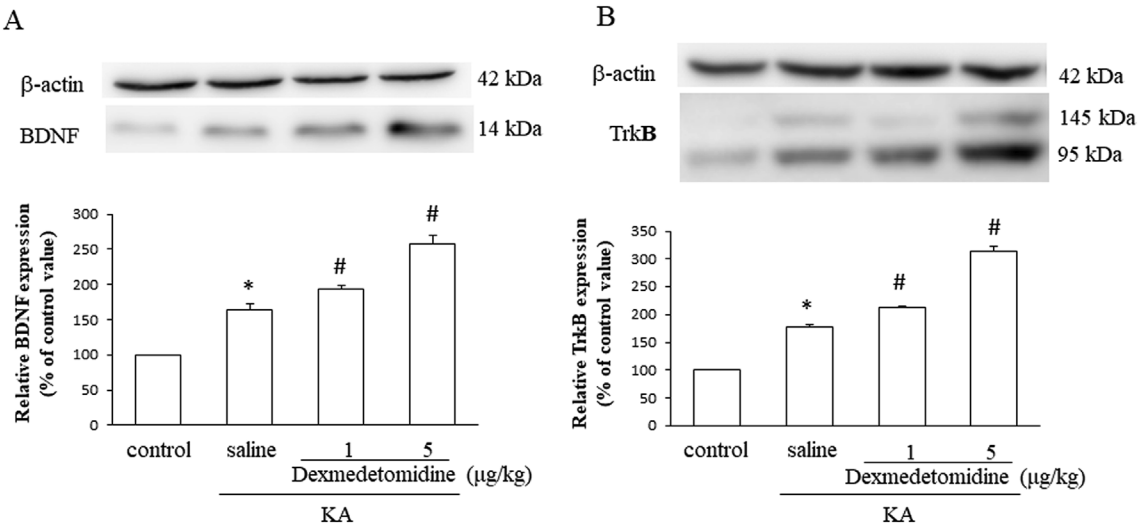

Fig. 3. Dexmedetomidine reversed KA-induced change in neurotrophic-factor protein expression in the hippocampus.

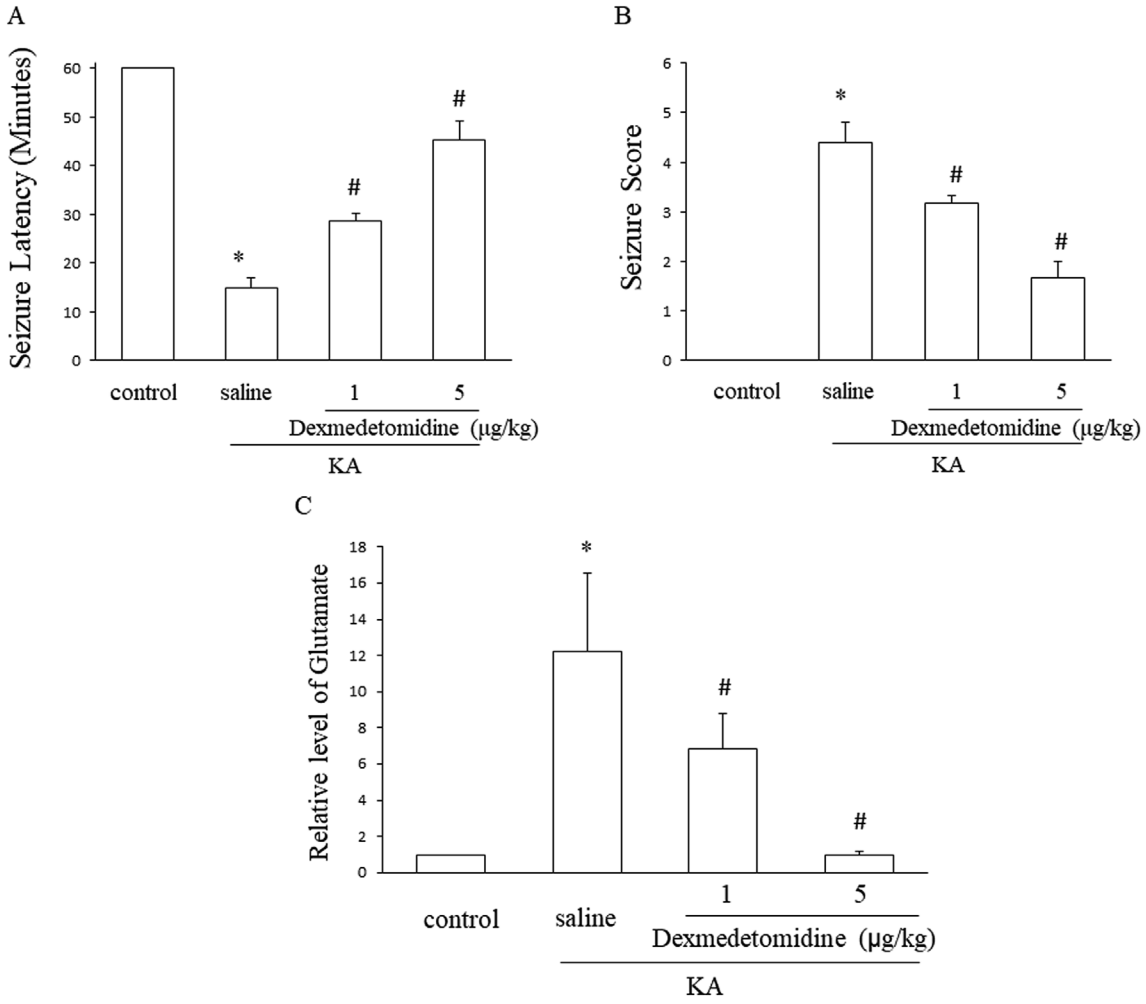

Fig. 4. Dexmedetomidine reduces both KA-induced seizure behavior and glutamate release in rat hippocampi.

in which dexmedetomidine have an *in vivo* anticonvulsant action on seizures induced by convulsant drugs or by an electrical stimulation (Kan et al., 2013; Tanaka et al., 2005). Furthermore, prominently increased extracellular levels of glutamate was detected in KA-injected rats, and this was suppressed by dexmedetomidine pretreatment.

Systemic KA injection produces selective neuronal cell death with apoptotic features in the CA3 region of the hippocampus (Chiu et al., 2018; Weiss et al., 1996). In this study, TUNEL staining revealed that KA induces neuronal cell death in the CA3 region of the hippocampus, which is consistent with the findings of other studies (Lee et al., 2010; Park et al., 2008). Dexmedetomidine pretreatment reduced this KA-induced neuronal apoptosis, suggesting that dexmedetomidine acts as a potent neuroprotective agent in addition to being an anticonvulsant agent. Caspase-3, a critical apoptotic executor, has been implicated in hippocampal neuronal apoptosis induced by KA (Faherty et al., 1999). Additionally, research has indicated that the antiapoptotic protein Bcl-2 protects against seizure-induced neuronal death by suppressing caspase-3 activity. Our data revealed that KA induced neuronal apoptosis in rat hippocampi by increasing the levels of proapoptotic caspase 3 and reducing the expression of antiapoptotic Bcl-2. This phenomenon was reversed by pretreatment with dexmedetomidine. As far as the expression of the protein is concerned, immunohistochemistry shows the greater advantage of analyzing distribution and localization of caspase 3 and Bcl-2 protein in hippocampus. On the other hand, Western blot show complementary characteristics, providing quantitative determination of caspase 3 and Bcl-2 in hippocampus tissue lysate. Our results strongly support that the neuroprotective effect of dexmedetomidine involves the blockade of well-characterized caspase 3 apoptotic pathways. Additionally, it is well established that MAPKs play a critical role in KA-induced neuronal damage. According to previous studies, KA administration increased the activation of MAPKs, including ERK 1/2, p38, and JNK, in rat hippocampi (Lin et al., 2013). In the present study, JNK and p38 activation was observed at 1 h after KA treatment, but ERK 1/2 activation was lasted for up to 3 h, which are consistent with previous research (Jeon et al., 2000). Furthermore, we found that dexmedetomidine pretreatment reduced the KA-induced phosphorylation of ERK 1/2, p38, and JNK in rat hippocampus. Thus, these data suggest that a decrease in KA-induced caspase-3 activation and phosphorylation of MAPKs are involved in the mechanism through which dexmedetomidine exerts neuroprotective effects.

BDNF and its receptor, TrkB, play a critical role in the process by which KA induces neuronal damage (Khaspekov et al., 2004; Tandon et al., 1999). BDNF exerts its neuroprotective effect through pro-survival mechanisms, regulating hippocampal synaptic plasticity and neurogenesis (Nagahara and Tuszynski, 2011; Zuccato and Cattaneo, 2009). Here, we observed that a significant increase in BDNF and TrkB levels in the hippocampus 24 h after KA injection, which is consistent with other studies (Gleeson et al., 2010; Rudge et al., 1998; VonDran et al., 2014). Notably dexmedetomidine pretreatment induced an even greater expression in BDNF and TrkB in the hippocampus of KA-treated rats. After KA administration, many hippocampal pyramidal neurons of rats go on to die in spite of the upregulation of BDNF. The probable explanation for this phenomenon is that the increase in BDNF after KA treatment is not enough to provide protection to compromised neurons.

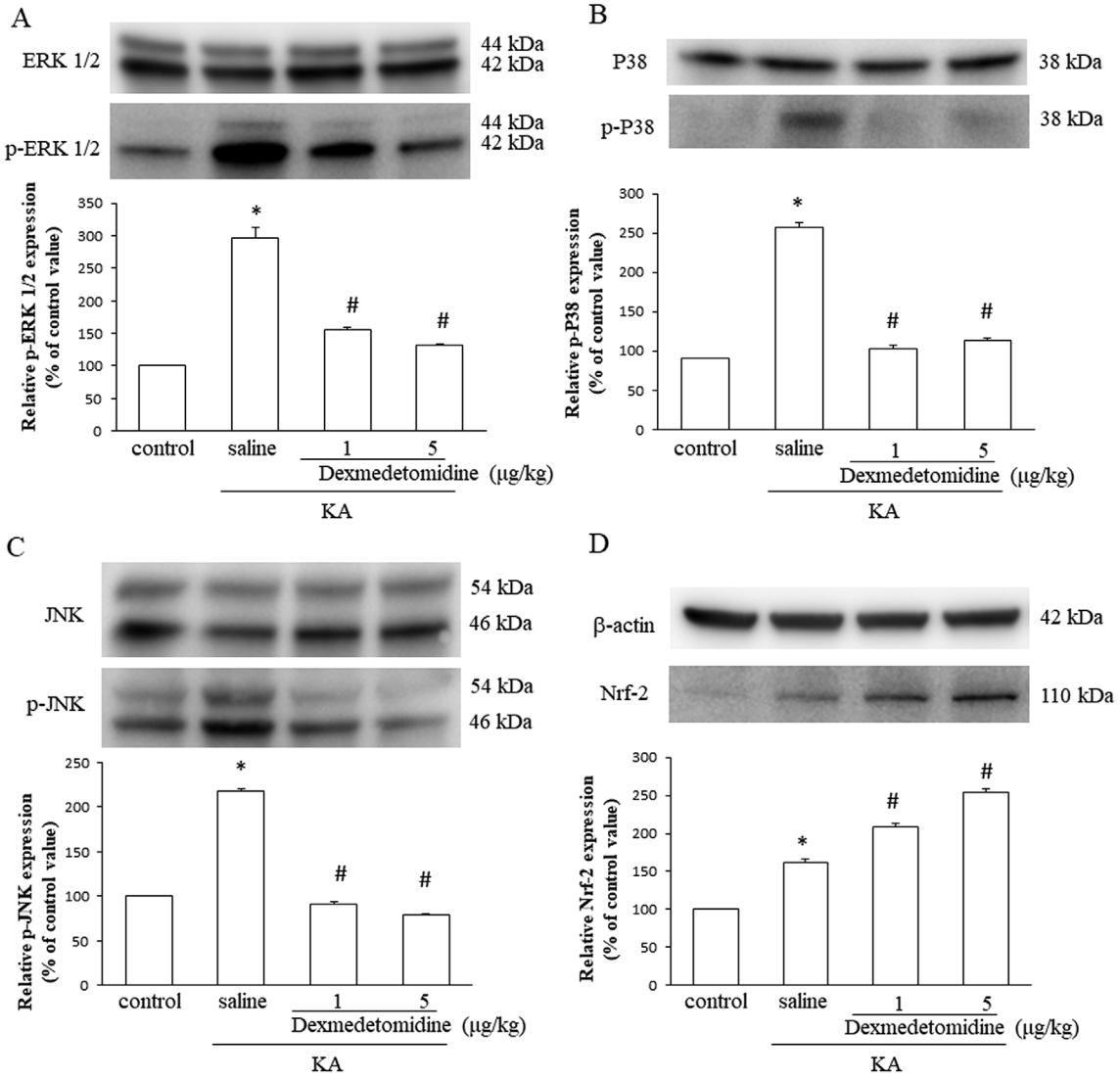

Fig. 5. Dexmedetomidine reduces KA-induced phosphorylation of mitogen-activated protein kinases and promotes expression of nuclear factor-erythroid 2-related factor 2.

Therefore, dexmedetomidine promote more BDNF expression in the hippocampus, which could be a potential mechanism for its neuroprotective effects against KA-induced excitotoxicity insult. On the other hand, the neuroprotective effect of BDNF is mediated via activating Nrf2 (Ishii et al., 2019), which is a transcription factor that regulates expression of many cytoprotective factors (Tufekci et al., 2011). In the present study, dexmedetomidine also increased the expression of Nrf2 in the hippocampus of KA-treated rats. These findings provide evidence that dexmedetomidine protects neurons against KA-induced neuronal apoptosis, and this neuroprotective effect may be closely related to an elevation in the level of the hippocampal BDNF/TrkB and Nrf2.

5. Conclusions

The results suggest that dexmedetomidine has significant anticonvulsant and neuroprotective effects in KA-treated rats, which constitutes a well-characterized model of temporal-lobe epilepsy. The neuroprotective effects may be caused by a reduction in glutamate levels, caspase-3 activation suppression, MAPK phosphorylation reduction, and the Nrf2 pathway and BDNF/TrkB signaling pathway activation in the hippocampus. Our investigation enrichens understanding regarding dexmedetomidine activity in the brain and demonstrates the therapeutic potential of this α2 adrenoceptor agonist for treating epilepsy and neurological disorders associated with excitotoxicity.

Disclosure statement

The authors have no conflicts of interest to disclose.

Funding sources

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The rats were pretreated with either saline or dexmedetomidine (1 or 5 μg/kg, i.p.) 30 min prior to KA (15 mg/kg, i.p.) injection. The extent of neuronal loss in the hippocampi was evaluated 3 days after KA injection by staining with (A1–A4) neutral red, (B1–B4) Fluoro-Jade B, and (C1–C4) TUNEL. The representative photomicrographs illustrate neuronal cell death in the hippocampal CA3 region of the (A1, B1, and C1) control, (A2, B2, and C2) KA, (A3, B3, and C3) KA plus dexmedetomidine 1 μg/kg, and (A4, B4, and C4) KA plus dexmedetomidine 5 μg/kg cases. Scale bar for A1-B4 = 100 μm and C1–C4 = 50 μm. (D) Quantification of Fluoro-Jade B-positive neurons in the CA3 region of the hippocampus. Results are presented as a mean ± SEM for 10 independent experiments. (E) Quantification of TUNEL-positive cells in the hippocampus. Results are presented as a mean ± SEM of seven rats

per group. *$P < 0.001$ vs. the KA group.

Rats were injected with the control, KA-treated, or KA plus dexmedetomidine (1 or 5 μg/kg) dosages and sacrificed 3 days after KA injection. Representative photomicrographs illustrating (A1–A4) cleaved caspase-3 and (C1–C4) Bcl-2 immunoreactivity in the hippocampi of the (A1 and C1) control, (A2 and C2) KA-treated, (A3 and C3) KA plus dexmedetomidine 1 μg/kg, and (A4 and C4) KA plus dexmedetomidine 5 μg/kg subjects. Representative images from six independent experiments are presented. Scale bar, 100 μm. Western blot and quantification of cleaved (B) caspase-3 and (C) Bcl-2 expression in the hippocampus from differently treated groups. Data are presented as a mean ± SEM of three rats per group, expressed as a percentage of control values. *$P < 0.001$ for comparison with the control group; #$P < 0.001$ for comparison with the KA-treated group.

Western blot analyses and the quantification of (A) BDNF, and (B) TrkB protein expression in the hippocampus from the control, KA-treated, KA plus dexmedetomidine (1 μg/kg), and KA plus dexmedetomidine (5 μg/kg) groups. β-actin was used as the internal control. Results are presented as mean ± SEM. *$P < 0.05$ for comparison with the control group; #$P < 0.05$ for comparison with the KA-treated group.

The rats were pretreated with saline or dexmedetomidine (1 or 5 μg/kg, i.p.) 30 min prior to KA (15 mg/kg, i.p.) injection. Seizure-behavior tests, including those on (A) latency of seizure onset and (B) severity of KA-induced convulsions, were performed 1–4 h after KA administration, according to the methods. The levels of glutamate in the hippocampi were evaluated using HPLC 3 days after KA injection (C). The bars indicate mean ± SEM. The number of animals in each group was 10. *$P < 0.05$ vs. the control group. #$P < 0.005$ vs. the KA group.

Western blot and quantification of the levels of phosphorylated (A) ERK 1/2, (B) p38 and (C) JNK relative to total ERK 1/2, p38 and JNK in the hippocampus from the control, KA-treated, KA plus dexmedetomidine (1 μg/kg), and KA plus dexmedetomidine (5 μg/kg) groups. The protein level of (D) Nrf2 was also investigated by Western blot. β-actin was used as the internal control. Results are presented as mean ± SEM. *$P < 0.0001$ for comparison with the control group; #$P < 0.001$ for comparison with the KA-treated group.

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