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Anticonvulsant effect of dexmedetomidine in a rat model of self-sustaining status epilepticus with prolonged amygdala stimulation

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HIGHLIGHTS

- Alpha2-adrenoreceptor served as a novel target to alleviate status epilepticus.
- Dexmedetomidine attenuated the severity of amygdala kindled status epilepticus.
- The mechanism related to the decreased level of Glu, while not to the GABA.
- Another mechanism attributed to the antioxidation of dexmedetomidine.

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ABSTRACT

Status epilepticus (SE), leading to 27 percent mortality in adult patients, becomes refractory to first-line intravenous diazepam, with prolonged seizure duration. The mechanism could be attributed to the declined inhibitory action of GABA; therefore, alternative medications acting on other targets are necessary. The aim of the present study was to examine whether DEX, a highly specific central α2-adrenoreceptor agonist, could show the anticonvulsant effect on self-sustaining SE (SSSE), and to explore the involved mechanisms. Five minutes after SSSE, which was induced in adult Wistar rats by constant amygdala stimulation for 25 min, DEX was injected intraperitoneally at two dosages (50/100 μg/kg). The number and cumulative time of repeated seizures were recorded; the levels of Glu/GABA and glutathione/malondialdehyde (GSH/MDA) in hippocampus tissue were detected. The results showed that DEX effectively decreased the number and cumulative time of repeated seizures, alleviated the levels of Glu and GSH/MDA in hippocampus tissue, but no effect was detected on the level of GABA, suggesting that DEX could be a potential agent for the treatment of SSSE, the possible mechanisms were antioxidation and inhibition of the Glu release.

1. Introduction

Status epilepticus (SE) is an intractable pathophysiological process with high mortality (about 27%) [17]. In clinical practice, intravenous diazepam alone or together with phenytoin, fosphenytoin and propofol is the standard first-line therapy, and the mechanisms are associated with inhibitory neurotransmitter GABA [19,20]. In some cases, however, SE rapidly becomes refractory with prolonged duration [10], and an increased dosage of medication has been associated with untoward side effects, such as dose-dependent respiratory suppression and the change of hemodynamics [20]. Given the formidable high mortality of patients with refractory SE, alternative treatments acting on different targets are needed to effectively alleviate the pathophysiological process.

Previous study has demonstrated that α2-adrenoceptor existing within central nervous system may play an important role in seizure suppression [2]. For instance, clonidine, an antihypertensive agent, can perform suppressive effect on seizure by selectively acting on central α2-adrenoceptor in pentylenetetrazol-induced rat seizure model [16]. However, the relatively low selectivity to α2-adrenoceptor and concomitant selectivity to α1-adrenoceptor make clonidine not widely accepted. Dexmedetomidine (DEX), a highly specific central α2-adrenoceptor agonist with minimal influence on respiration and hemodynamics [8], has been used for anesthesia, sedation and analgesia in clinical practice. Report has revealed that DEX can increase the convulsive threshold in cocaine-injected rats [27], but it is unknown whether DEX can perform beneficial effect on SE. Amygdala kindling is a recognized temporal lobe epileptic model, which can progress to generalized

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seizure [14]. Report has shown that after applying high-intensity (700 μA) pulsed-train electrical stimulation focusing on the basolateral nucleus of amygdala (BLA) for 25 min, a self-sustaining SE (SSSE) can be induced in Wistar rat [3]. The aim of the present study was to examine the effect of DEX on SSSE in a rat model with prolonged amygdala stimulation, and to explore the involved mechanisms.

2. Materials and methods

2.1. Animals

Adult male Wistar rats weighing 250–300 g ($n = 65$) were obtained from the Hebei Medial University. The rats were housed in groups of five per cage in a room with constant temperature ($25 \pm 1\,^{\circ}C$) and humidity (40–60%), and were kept on a 12 h light/dark cycle, with lights on at 8:00 AM and with free access to food and water.

Animal experiments were performed according to the regulations of laboratory animal management promulgated by the Ministry of Science and Technology of the People's Republic of China [1988] No. 134, which coincides with internationally recognized NIH guidance for care and use of laboratory animals. All efforts have been made to minimize suffering and to use minimal numbers of animals with sufficient power of results.

DEX was purchased from Jiangsu Hengrui medical limited company (China). GSH detection kit and MDA detection kit were obtained from Nanjing Jiancheng Bioengineering Institute (China). DEX was diluted from 200 to 20 μg/ml with normal saline prior to the experiments.

Animals were randomly divided into five groups: Group I (SSSE plus low dosage DEX): intraperitoneally DEX (50 μg/kg)-injected and electrically stimulated animals ($n = 15$); Group II (SSSE plus high dosage DEX): intraperitoneally DEX(100 μg/kg)-injected and electrically stimulated animals ($n = 15$); Group III (SSSE): electrically stimulated and non-DEX-injected animals ($n = 15$); Group IV (sham): electrode-implanted but non-DEX-injected and non-electrically-stimulated animals ($n = 10$); and Group V (control): animals without any treatment ($n = 10$).

2.2. Electrode implantation

After being anaesthetized with 10% chloraldurate (0.4 g/kg), rats in Group I–IV were stereotactically implanted with twisted stainless steel wire bipolar stimulating electrodes in the left BLA according to the rat brain atlas of Paxinos and Watson (1998) (BLA; Bregma: anterior: −2.8 mm; lateral: 4.8 mm; ventral: 8.8 mm from skull surface). Three screws were positioned over the right frontal cortex, left and right occipital cortices served as recording, reference and ground electrodes. Dental cement was used to bind the electrodes and screws to the skull. ABS connectors coupled the electrodes and screws to stimulating and recording equipments.

2.3. Induction of self-sustaining status epilepticus

Electrical stimulations of the BLA were initiated following a recovery period of at least 2 weeks after electrode implantation. The afterdischarge (AD) threshold was determined by stimulating the left BLA, once every minute, with 1 s, 60 Hz monophasic square-wave pulses of ascending intensity (started with 10 μA base-to-peak, then raised by 20% every time) until an AD was elicited. An AD was defined as a spike train that outlasted the electrical stimulus by 3 s or more. After detection of AD threshold in Group I–III, a kindling protocol in BLA was performed once a day by constant current stimulation, which was higher than all the determined AD thresholds. The parameters were 500 μA, 1 ms, monophasic square-wave pulses, and 60 Hz for 1 s. The seizure stage was evaluated according to Racine. Stage 1: immobility and facial automatisms (eye closure and facial clonus); stage 2: head nodding and more severe facial and mouth clonus (mastication); stage 3: unilateral forelimb clonus; stage 4: rearing and bilateral forelimb clonus; stage 5: rearing and falling accompanied by generalized tonic–clonic seizures. The rats were not considered kindled until at least 5 consecutive seizures of stage 5 were elicited. About 2 weeks after the last kindling, the prolonged stimulation of the ipsilateral BLA for induction of SSSE was performed as below: stimulus duration of 25 min and stimulus program consisting of 100 ms trains of 1 ms square-wave pulses. The trains were given at a frequency of 2/s and the intra-train pulse frequency was 50/s. Peak pulse intensity was 700 μA. The beginning of SSSE was determined as the occurrence of high-amplitude and high-frequency discharge (HAFD) after the cessation of stimulation, which was the conspicuous feature of EEG during SSSE, and was defined as high-amplitude (>2× baseline), high-frequency (>8 Hz), and lasting for at least 5 s. The end of HAFD was marked by a brief (1–3 s) "flat period" on the EEG [11]. Five minutes after the emergence of induced SSSE, the rats in Group I and II were injected intraperitoneally with DEX (50/100 μg/kg) to examine whether DEX could alleviate SSSE. Development and duration of SSSE (i.e. the time between the first and the last HAFD) were monitored for 12 h by EEG through a powerlab data acquisition system (AD Instruments, AUSTRALIA). The number of discrete seizures and the cumulative time (duration of SSSE subtracting interictal time) of seizures were recorded for comparison of SSSE severities [26]. Offline analyses of the EEG recordings were performed using Labchart Pro software (AD Instruments, AUSTRALIA) to measure the cumulative time of seizures. In Group IV and V, the rats were not subject to the electrical stimulation.

Twelve hours after the stimulation, all rats were sacrificed and the hippocampus tissues in brains were removed, cleaned with ice-cold saline, and frozen in liquid nitrogen immediately. All samples were stored in $-80\,^{\circ}C$ refrigerator until detecting. Hippocampus samples were thawed and 10% (w/v) homogenates were made with ice-cold 0.1 M phosphate buffer (pH 7.4), and were used to determine the content of glutamate (Glu), an excitatory amino acid, and GABA, an inhibitory amino acid, by the liquid chromatography–tandem mass spectrometry (LC–MS/MS) which consisted of an Agilent 1200 liquid chromatography system (USA) equipped with a quaternary solvent delivery system, an autosampler and a column compartment and a 3200 QTRAP™ system (Applied Biosystems, Foster City, CA, USA) with a hybrid triple quadrupole linear ion trap mass spectrometer equipped with Turbo V sources and Turbolonspray interface.

The same homogenates were also used to determine lipid peroxidation and glutathione (GSH). Malondialdehyde (MDA), reflecting lipid peroxidation, was measured spectrophotometrically with the method described by Okhawa [12] and GSH was measured spectrophotometrically with the method described by Ellman [6].

All values were expressed as mean ± S.E.M. Statistical analyses were made with the aid of SPSS 10.0 for Windows. Statistical comparisons were conducted using one-way ANOVA followed by LSD and SNK tests to determine significance among individual groups. Differences were considered significant if $p < 0.05$.

3. Results

Thirteen rats in Group I, 11 rats in Group II, and 15 rats in Group III were kindled during the amygdala kindling protocol. Two weeks after the protocol, SSSE, defined as continuous intermittent HAFDs recorded by EEG lasting at least 15 min (as shown in Fig. 1), was induced in all kindled rats by continuously