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Research Paper

Rapid focal cooling attenuates cortical seizures in a primate epilepsy model

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ABSTRACT

Rapid focal cooling is an attractive nondestructive strategy to control and possibly prevent focal seizures. However, the temperature threshold necessary to abort seizures in primates is still unknown. Here, we explored this issue in a primate epilepsy model and observed the effect of rapid cooling on different electroencephalogram frequency bands, aiming at providing necessary experimental data for future clinical translational studies and exploring the mechanism of focal cooling in terminating seizures. We induced focal neocortical seizures using microinjection of 4-aminopyridine into premotor cortex in five anesthetized cynomolgus monkeys. The rapid focal cooling was implemented by using a thermoelectric (Peltier) device. As a result, the average durations of seizures and interictal intervals before cooling were 94.3 ± 4.0 s and 62.3 ± 6.9 s, respectively. When the cortex was cooled to 20 °C or 18 °C, there was no effect on seizure duration (109.4 ± 30.0 s, 91.3 ± 19.3 s) or interictal duration (99.4 ± 26.8 s, 83.2 ± 11.5 s, $P > 0.05$). But when the cortex was cooled to 16 °C, the seizure duration was reduced to 54.1 ± 4.9 s and the interictal duration was extended to 175.0 ± 16.7 s ($P < 0.05$). Electroencephalogram spectral analysis showed that the power of delta, alpha, beta, gamma and ripples bands in seizures were significantly reduced at 20 °C and 18 °C. At 16 °C, the power of theta band in seizures was also significantly reduced along with the other bands. Our data reveal that the temperature threshold in rapid focal cooling required to significantly shorten neocortical seizures in nonhuman primates is 16 °C, and inhibition of electroencephalogram broadband spectrum power, especially power of theta band, may be the underlying mechanism to control seizures.

1. Introduction

To date, the first choice for treatment of epilepsy is still antiepileptic drug therapy. However, despite the availability of over 20 antiepileptic drugs, seizures still remain uncontrolled in about 30% of patients (Kwan et al., 2010; Kwan et al., 2011). In recent years, surgical approaches are fast developing, but for patients with epileptogenic foci localized in the functional cortex or in multiple areas, they have to look for alternative therapeutic methods.

In past decades, endovascular cooling and external cooling has been used clinically for the treatment of refractory status epilepticus and postanoxic epilepsy (Corry et al., 2008; Low et al., 2012; Ren et al., 2015; Srinivasakumar et al., 2013). However, the extensive systemic complications of endovascular and external cooling limit its uses and effectiveness. Furthermore, rapid focal cooling is also proposed as an attractive strategy to treat focal seizures. It's reported that focal infusion of cold saline on the neocortex, ventricle and subarachnoid space could successfully treat intractable epilepsy and reduce intraoperative stimulation induced seizures (Karkar et al., 2002; Ommaya and Baldwin, 1963; Sartorius and Berger, 1998; Sourek and Travnicek, 1970). In addition, preclinical research has also demonstrated that rapid focal cooling can quickly control seizures or epileptic discharges both in vitro and vivo (Burton et al., 2005; Hill et al., 2000; Imoto et al., 2006; Moseley et al., 1972; Motamedi et al., 2006; Tanaka et al., 2008; Thompson et al., 1985; Vastola et al., 1969; Yang et al., 2002; Yang and Rothman, 2001).

Abbreviations: EEG, electroencephalogram; 4-AP, 4-aminopyridine; GFAP, glial fibrillary acidic protein; TUNEL, TdT mediated dUTP-biotin nick-end labeling.

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In the rodent epilepsy model, when the focal cortical temperature is rapidly lowered to 20–24 °C with a small Peltier device, seizures can be controlled within a few seconds (Moseley et al., 1972; Yang et al., 2002; Yang and Rothman, 2001), and the subsequent seizure frequency and intensity are markedly reduced (Yang et al., 2003; Yang and Rothman, 2001). Some studies also found that neocortical or hippocampal epileptic discharges could be inhibited by lowering the focal temperature to 15–23 °C (Fujii et al., 2012; Imoto et al., 2006; Tanaka et al., 2008). No histological changes related to focal cooling have been reported even with target temperatures as low as 0–5 °C in some experiments (Oku et al., 2009; Yang et al., 2006). Therefore, rapid focal brain cooling is a strikingly attractive, nondestructive strategy to be applied clinically to control and possibly prevent focal seizures (Smyth and Rothman, 2011).

So far, previous studies of rapid focal cooling in controlling seizures are mostly done on rodents. Because the brain of rodents and human beings are significantly different in the size and anatomy, the experimental data for rapid focal cooling in termination of seizures must be obtained in large animal epilepsy models, such as primates. Currently, the number of experiments using primate models is limited. We have demonstrated that small Peltier devices have sufficient power to cool the cortex of rhesus monkeys, and we have explored the target temperature needed to influence finger movement (Steven and Marc, 2005). Another study of patients with intractable epilepsy focused on the effect of focal cooling on cerebral blood flow and transmitter metabolism, but they did not assess for temperature thresholds needed to control seizures (Nomura et al., 2014). The threshold temperature needed to control seizures is an important parameter for conducting translational studies, and it determines the supplied power for cooling and strength for heat dissipation of an implantable cooling device for human beings in the future.

To address this problem, we conducted an experiment in cynomolgus monkeys to explore the threshold temperature of cooling necessary to abort seizures in primates, the effect of focal cooling on disparate frequency-bands, and the mechanism by which focal cooling controls seizures. This is the first study directly aimed at exploring the threshold temperature needed to control seizures in primates, which will vigorously promote the clinical translational study of therapeutic rapid focal brain cooling to treat intractable focal epilepsy.

2. Materials and methods

2.1. Neocortical seizure model

We used a protocol approved by Capital Medical University and Wincon TheraCells Biotechnologies Limited Company Animal Studies Committees. Five 5–7 years old (8–9 kg), adult male cynomolgus monkeys were used in this study. Before moving out from the cage, the monkeys were intramuscularly injected with an initial anesthetic agent (ketamine, xylazine and midazolam, 0.15 ml/kg). After tracheal intubation, the monkeys were anesthetized with halothane and placed on a heating pad in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, U.S.A.). We administered the halothane continuously through a tube connected to the anesthetic machine. The concentration of halothane was 1% while a craniotomy was performed, but was reduced to 0.5% when we induced seizures. An intravenous line was set up for fluid infusion.

Under the condition of sterile operation, a round cranial window (diameter: 15 mm) was created over the premotor cortex using an orthopedic drill. The center of the cranial window was located in the premotor cortex area correlated with hand movement (anterior-posterior from the ear bars: 30 mm, medial-lateral: 14–15 mm). Then a 5 × 3 mm rectangular cranial window was made after the round window to contain the thermoelectric power lines. At both sides of the rectangular skull window, we placed two screws symmetrically to fix the cooling device (Fig. 1A). During the drilling, the skull was continuously irrigated with artificial cerebrospinal fluid to prevent the underlying brain from overheating. After creation of the window, the dura and pia were gently opened to allow drug injection and direct application of the Peltier device to the brain surface.

We induced recurrent, focal neocortical seizures by injecting 30 μl of 4-aminopyridine (4-AP) solution (25 mM in artificial cerebrospinal fluid) using a commercial manual microsyringe pump (SGE100TLL, WPI, Sarasota, Florida, U.S.A.) coupled to a special needle (tip diameter, 600 μm). The injection system was mounted on a micromanipulator that allowed us to precisely control the injection of 4-AP into 3 mm below the cortex. The injection was carried out over a 10-minute period to minimize cortical trauma, and the needle was left in place until the end of the experiment to prevent the leakage of 4-AP from the injection site.

2.2. Rapid focal cooling

We focally cooled the neocortical surface with a commercially available ring-shaped thermoelectric (Peltier) device (TE technology, Traverse City, MI, U.S.A. Fig. 1B). Its outer diameter, inner diameter, and thickness are 15 mm, 3 mm, and 3.6 mm, respectively. The ring-shaped thermoelectric chip was glued to the bottom of an oval hollow copper heat sink. The heat sink (15 × 25 × 5 mm) had one pair of water inlet and outlet that were connected to a peristaltic pump through the silicone tubes. We perfused the cold saline (0–4 °C, 90 ml/min) into the heat sink to achieve the purpose of the radiator. We fixed the whole cooling device to the skull using screws through symmetrical holes on both sides of the heat sink allowing the Peltier device to touch just the surface of the neocortex. The whole cooling process was controlled by our laboratory-designed temperature control system. The temperature of the cortex surface was monitored by attaching two 0.13 mm-diameter thermocouples (Spectris, Surrey, England, UK). One was connected to an electroencephalogram (EEG) recording instrument to synchronously and continuously track the temperature, and the other was connected to the temperature control system to provide feedback and adjust the cooling process. The power that we used to supply the Peltier device was 1.9 V.

Based on our previous experimental results obtained from rat models (Yang and Rothman, 2001), we set three temperature targets for cooling: 20 °C, 18 °C and 16 °C. For the purpose of using as few experimental animals as possible, we took the seizures before cooling of each monkey as the control group (self-controlled). Because of the long-lasting effects of cooling, we did not apply subsequent cooling until seizures had returned to the control condition. Furthermore, we randomly assigned a set sequence of the three target temperatures for each monkey. Each time, cooling lasted for 1 min, which is shorter than the shortest duration of control seizures.

To confirm the effects of rapid focal cooling at different depths below the neocortex, we mapped the temperature gradient with a needle thermocouple (diameter: 0.2 mm, Spectris, Surrey, England, UK). The thermocouple has one contact at the tip and was inserted into the cortex through the central hole of the ring-shaped Peltier device at different depths. Then, we measured the actual temperature at 1, 2, 3, and 4 mm below the depth of the cortex when the cortical surface temperature was reduced to 20 °C and 15 °C, respectively.

2.3. Electroencephalography

We placed two screw electrodes symmetrically in the frontal lobes over each hemisphere as positive pole, one screw electrode in occipital lobe as negative pole and one reference electrode in the subcutaneous muscle to record subdural EEG. Besides, one depth electrode was placed 3 mm below the cortex at the injection site of 4-AP to record intracortical EEG (Fig. 1A). The EEGs were recorded and digitized using a 16-channel Powerlab system (AD instrument, Springs, Colorado, U.S.A.) with a sampling rate of 1000 Hz. The EEG was monitored 30 min before the injection of the 4-AP and continued throughout the entire experiment.