Effective suppression of hippocampal seizures in rats by direct hippocampal cooling with a Peltier chip

Laboratory investigation

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Object. The use of focal brain cooling to eliminate epileptic discharges (EDs) has attracted increasing attention in the scientific community. In this study, the inhibitory effect of selective hippocampal cooling on experimental hippocampal seizures was investigated using a newly devised cooling system with a thermoelectric (Peltier) chip.

Methods. A copper needle coated with silicone and attached to the Peltier chip was used for the cooling device. The experiments were performed first in a phantom model with thermography and second in adult male Sprague–Dawley rats in a state of halothane anesthesia. The cooling needle, a thermocouple, and a needle electrode for electroencephalography recording were inserted into the right hippocampus. Kainic acid (KA) was injected into the right hippocampus to provoke the EDs. The animals were divided into hippocampal cooling (10 rats) and noncooling (control, 10 rats) groups.

Results. In the phantom study, the cooling effects (9°C) occurred in the spherical areas around the needle tip. In the rats the temperature of the cooled hippocampus decreased below 20°C within a 1.6-mm radius and below 25°C within a 2.4-mm radius from the cooling center. The temperature at the needle tip decreased below 20°C within 1 minute and was maintained at the same level until the end of the cooling process. The amplitude of the EDs was suppressed to 68.1 ± 4.8% of the precooling value and remained low thereafter. No histological damage due to cooling was observed in the rat hippocampus.

Conclusions. Selective hippocampal cooling effectively suppresses the KA-induced hippocampal EDs. Direct hippocampal cooling with a permanently implantable system is potentially useful as a minimally invasive therapy for temporal lobe epilepsy and therefore could be an alternative to the temporal lobectomy.

(Key Words: • focal cooling • hippocampal seizure • kainic acid • rat • thermoelectric device)

Temporal lobe epilepsy is a well-known type of epilepsy that can be successfully treated with surgical procedures such as an anterior temporal lobectomy or selective amygdalohippocampectomy.2,11 Reportedly, however, left-sided temporal lobectomy for TLE is often complicated by memory decline and language dysfunction.2,9,11,19,20,23,24,26 Some cases of psychosis arising de novo after a temporal lobectomy have also been reported.5,23 Furthermore, in patients who have undergone an anterior temporal lobectomy, superior quadrantopia occasionally occurs due to an injury to the Meyer loop.5 It is therefore essential to develop minimally invasive procedures for the treatment of TLE.

Brain cooling has been well established as an effective method of suppressing EDs.4,14,16,18,25,27,30 In an experimental model of neocortical epilepsy, we demonstrated the suppression of EDs by focal cortical cooling, which was induced by a Peltier chip.15 Furthermore, focal cortical cooling that terminates EDs has no discernible effect on normal cortical functions.4,17,31,34 In contrast to the case for neocortical epilepsy, however, the effects of focal cooling on seizures arising from the hippocampus (that is, hippocampal seizures) have not been thoroughly investigated, chiefly because of the difficulties in selective cooling of the hippocampus.

In this study, we investigated the inhibitory effect of focal cooling on experimental hippocampal seizures by using a newly devised cooling system with a Peltier chip.15,34 In this system, a copper needle for direct insertion into the hippocampus is attached to the chip, thereby allowing direct hippocampal cooling. The demonstration of such an inhibitory effect with a permanently implantable cooling system might signify the advent of a new minimally invasive therapy that could replace the temporal lobectomy.
Materials and Methods

Cooling Device

We used a Peltier chip as the thermoelectric device (Fig. 1). The chip (6 × 6 mm, with a thickness of 2 mm) was composed of 2 conductors, which were connected in parallel. When an electric current passed between the conductors, 1 side was cooled and the other was heated because of an electronic refrigeration phenomenon. To reduce the temperature of the heated side, we attached a heat sink to the chip to help dissipate the heat generated. This heat sink was made of copper and had a water channel inside. Two silicone tubes were connected to the heat sink, and cold water (4°C) was pumped into and circulated in the channel. A cooling needle (length 6 mm, external diameter 1 mm) and a plate, both of which were made of copper, were attached to the Peltier chip. The tip of the needle was exposed, and the remainder of the needle was coated with the silicone. These devices were designed to cool the hippocampus directly and to maintain cooling efficiency. A thermometer was also attached to the device.

General Preparation

All of the experiments were performed according to the Guidelines for Animal Experimentation of the Yamaguchi University School of Medicine. Adult male Sprague–Dawley rats, each weighing between 350 and 450 g, were used. After inducing anesthesia with a mixture of 30% nitrous oxide, 66% oxygen, and 4% halothane, a breathing tube was inserted, mechanical ventilation was begun, and the halothane concentration was subsequently kept at 0.5–1%. A femoral artery and a femoral vein were cannulated for continuous monitoring of arterial blood pressure, obtaining blood samples, and administering drugs. Animals were then placed prone, fixed in a stereotactic frame (Narishige Co.), and immobilized with the intravenous administration of pancuronium bromide (4 mg/kg).

Rectal temperature was monitored using a thermometer and maintained at 100–120 mm Hg. With the aid of an operating microscope, the left parietal skull was exposed, and a craniotomy (10 × 9 mm) was made to expose the dural arachnoid by using a high-speed saline-cooled dental drill after the application of 1% lidocaine to the skin.

Examination of Cooling Performance

First, we studied the cooling performance of the system in a phantom model with thermography. The cooling needle was inserted in the phantom material (agar), and the temperature distribution was recorded during cooling when 1 A of electric current was passed through the Peltier chip (Fig. 2).

Next, we determined the temperature distribution in the rat hippocampus. The cooling needle and thermometer were inserted into the left hippocampus at a depth of 4.0 mm, and the temperature was monitored using a thermometer and maintained at 37°C using a heating pad. Systolic blood pressure was maintained at 100–120 mm Hg. With the aid of an operating microscope, the left parietal skull was exposed, and a craniotomy (10 × 9 mm) was made to expose the dural arachnoid by using a high-speed saline-cooled dental drill after the application of 1% lidocaine to the skin.

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Effects of the Selective Hippocampal Cooling on Hippocampal Seizures

We used KA to provoke the EDs in accordance with methods reported in previous studies. A microinjection needle for the KA injection was placed in the right hippocampus at a depth of 4.0 mm through a small craniotomy (stereotactic coordinates relative to bregma: 4.0 mm posterior and 3.0 mm lateral; Fig. 3). Kainic acid was dissolved in 0.01 mol/L of phosphate-buffered saline (pH 7.4) and was adjusted to 1 mg/ml. One microliter of the solution was injected into the right hippocampus over a period of 10 minutes by using a microinjection pump (Eicom Co.) to avoid tissue injuries and leakage of the solution along the needle. In this model, the EDs were recorded in the left hippocampus after the KA injection into the right hippocampus.

The cooling device was fixed at the craniotomy site (Fig. 3). A reference electrode for EEG recording was inserted in the neck muscle, and the EEG activity of the left hippocampus was continuously monitored with a digital electroencephalograph (Unique Medical Co.) after the KA injection into the right hippocampus. The conditions for recording EEG activity were programmed as follows: time constant, 0.3 seconds; high-frequency filter, 60 Hz; and notch filter, on. Focal cooling was started 5 minutes after confirming the appearance of left hippocampus EDs. The EEG activity and temperature in the left hippocampus were recorded continuously for 20 minutes, from 1 minute before the start of cooling.

Animals were randomly divided into 2 experimental groups: the control group, in which the entire system was set but the hippocampal cooling was not induced (10 rats); and the cooling group, in which hippocampal cooling was induced by turning on the electric current to the Peltier chip (10 rats).

Histological Examination

After performing the cooling experiment, the rats in both the control and cooling groups were anesthetized deeply and killed by transcardial perfusion; they were first injected with heparinized saline at 100 mm Hg until the perfusate from the right atrium ran clear and then with 10% formaldehyde. The brain in each animal was removed and stored in 10% formaldehyde for at least 24 hours and routinely processed for paraffin embedding. Each brain, including the cooled areas, was cut into coronal sections measuring 3 μm thick. The sections were mounted on glass slides and stained with H & E.

Statistical Analysis

Data are expressed as the mean ± the standard error of the mean. Changes in the mean amplitude were tested using a repeated-measures analysis of variance. When significant differences were detected, the differences between the control and cooling groups, at the same time point, were tested using an unpaired t-test. The differences were considered to be statistically significant at a probability value < 0.05.

Results

Performance of the Cooling System

In the phantom study, thermography showed that the temperature at the tip of the needle reached ~ 9°C and that cooling effects occurred in the spherical areas around the needle tip (Fig. 2 right). The temperature distribution in the rat hippocampus is shown in Fig. 4. The temperature of the
cooled site in the hippocampus reached below 20°C within a 1.6-mm radius and below 25°C within a 2.4-mm radius from the cooling center.

**Changes in EDs by Hippocampal Cooling**

In the control group animals, in which hippocampal cooling was not induced, the EDs appeared in the left hippocampus within 30 minutes after the KA injection into the right hippocampus and did not disappear for 1 hour. In the cooling group animals, in which the electric current to the Peltier chip was turned on, the amplitudes of the EDs were decreased markedly during cooling and increased to precooling levels during the rewarming period (Fig. 5).

Figure 6 illustrates typical examples of the changes in the amplitudes and RMSs of the EDs and in the temperature at the needle tip. The temperature at the needle tip decreased below 20°C within 1 minute and was maintained at the same level until the end of the cooling process. The amplitude of the EDs gradually decreased after the start of cooling, and the RMS also decreased by ~50% at the end of cooling. The amplitude then remained low during the subsequent period.

Because the amplitude of the EDs varied among each rat, we calculated the mean amplitude of the discharges as well as the temperature every 30 seconds before and after the start of cooling. Sequential changes in the hippocampal temperature and the ED amplitude expressed as a percentage of the precooling baseline levels are shown in Fig. 7. The temperature of the cooling site (the tip of the needle) was maintained at 33.1 ± 0.7°C before cooling and then decreased to 14.5 ± 1.3°C. The mean amplitude of the EDs in the cooling group animals was suppressed to 68.1 ± 4.8% of the precooling value at the end of the cooling period and subsequently stayed at levels lower than those in the control group animals. Statistically significant differences between the groups were detected from 60 seconds after the start of cooling until 210 seconds after the cessation of cooling (p < 0.05).

**Histological Findings**

Typical histological findings in animals in the cooling and control groups are shown in Fig. 8. Although hippocampal injuries and bleeding due to the insertion of the cooling needle occurred, no apparent difference in the hippocampus was observed between the cooling and control group animals.

**Discussion**

In neocortical epilepsy, focal brain cooling has been reported to reduce EDs in both experimental models and humans. Using a hippocampal slice epilepsy model, Motamedi et al. demonstrated that cooling aborted 4-aminopyridine–induced EDs in the hippocampus. However, there are so few reports concerning the effects of focal cooling on hippocampal seizures in vivo. Burton and colleagues revealed the inhibitory effects of focal cooling on hippocampus-kindled seizures, although they cooled both the hippocampus and the cortex simultaneously. Our cooling system utilized the Peltier chip with a copper needle attached to the chip. Copper is an accessible and pliable material for these applications.
material and has high thermal conductivity. The copper needle also has the necessary hardness for implantation. We coated the needle with silicone except for its tip, which enabled selective and efficient hippocampal cooling as shown in this study in both the phantom model and rat hippocampus.

These results indicated that our device is sufficiently able to suppress EDs by lowering the hippocampal temperature below 25°C within a 2.4-mm radius from the cooling center. Our findings closely correlate with those of previous studies,15,31,34 which have demonstrated that the suitable temperature of the cortical surface for terminating seizures is 20–25°C.

The precise mechanisms for the antiepileptic effects of focal cooling remain to be established, although several possible mechanisms have been proposed (for example, the reduction of neurotransmitter release, the alternation of activation/inactivation kinetics in voltage-gated ion channels, and the slowing of catabolic processes).1,10,14,29 Using a 2-photon microscopy technique, Yang et al.33 demonstrated that one major neurophysiological effect of cooling in the brain was a reduction in presynaptic neurotransmitter release. Furthermore, Javedan et al.16 reported that in rat hippocampal slices, a moderate level of cooling (21°C) causes a reversible block in the network synchrony, which is required to generate EDs, without any blockage of synaptic transmissions.

As mentioned previously, our cooling device can suppress hippocampal EDs. When determining clinical applications, however, the influence of cooling on normal brain functions and histological characteristics should be considered. Using guinea pig hippocampal slices, Aihara et al.1 revealed that the neuronal activity of pyramidal neurons—such as synaptic function, neuronal excitability, and membrane properties—maintain reversibility after relatively long-term (> 90 minutes), mild (20–25°C), and even severe hypothermia (8–15°C). In our previous study, no apparent EEG changes were observed by cooling the non-epileptic rat cortex to 23°C for 3 minutes.15 Bakken and colleagues3 have also reported the preservation of brain function after cooling the cerebral cortex in humans. Karkar and associates17 have also asserted that the motor-evoked potential is not changed by the application of cold saline for the termination of EDs caused by cortical stimulation mapping. Regarding histological considerations, no apparent changes due to cooling were observed on histological examination of the hippocampus in our study. We have also confirmed that cooling at 20°C for 30 minutes causes no histological damage to the rat cortex.15 A recent report by Yang et al.32 has revealed that a rat neocortex intermittently cooled to

Before cooling

Cooling

Rewarming

Fig. 5. Tracings depicting typical examples of the EEG recordings obtained in the cooling group animals before and after cooling.
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**Figure 6.** Graphs showing typical examples of the changes in the amplitude and RMSs of the epileptiform discharges and the temperatures at the needle tip in the cooling group animals before and after cooling.

**Figure 7.** Graphs depicting sequential changes in the temperature and amplitude of the EDs before and after cooling. Black circles represent the control group (10 rats); black squares, the cooling group (10 rats); and black triangles, mean temperatures in the cooling group. *p < 0.05.
5°C for 2 hours shows no histological damage and that neurons in the rat cortex cooled to 3°C for 2 hours daily for 10 months are also preserved. On the basis of such studies and the fact that a cooling period as brief as 1 minute can terminate seizures, focal cooling to below 20°C for a short period may be acceptable. Considering these experimental and clinical results together, one can safely say that both brain function and structure tolerate considerable degrees of focal cooling.

Note, however, that for the practical use of the implantable hippocampal cooling system in humans, there are several issues that must be addressed. First, the extent of the cooling area necessary to prevent human hippocampal seizures should be determined. As shown in the present study, the thin cooling needle (1 mm in diameter) was sufficient for the rat hippocampal seizures. The smaller the needle is, the better. However, the seizure focus in the hippocampus in humans is known to be much broader than in rat. When considering the cooling system in clinical use, a thicker needle or multiple needles may be necessary to cool the seizure focus in the hippocampus in humans. Furthermore, the cooling area can be increased in size by decreasing the cooling temperature to below 20°C. Further study is needed to determine the appropriate needle size and its applications in humans. Second, the time required to reach the target temperatures should be shortened. As shown in this study, it takes 60 seconds to reach the target temperatures in the rat. Motamedi and associates²¹ have demonstrated that slow cooling (0.1–1°C/second) requires much greater temperature drops to inhibit the EDs than rapid cooling (2–5°C/second). Niederhauser and colleagues²² have reported that in TLE, patient-specific EEG events occurred 5–80 seconds prior to seizure onset. From this perspective (that is, EEG detection and reduction of the cooling time), the cooling system should be improved before its clinical application. Third, the materials of the implanting needle should be considered. In this study, copper was chosen because of its pliability, high thermal conductivity, and suitable hardness, as mentioned earlier. However, the use of other materials such as silver, which may be more innocuous to the living brain, should be considered. Fourth and last, the size of the components, such as the electric power supply, EEG detection system, and thermometer, should be minimized as much as possible for future clinical use. An implantable local cooling system in humans will likely become a reality with technological developments.

**Conclusions**

We confirmed that selective hippocampal cooling suppressed the EDs induced by KA injection into the contralateral hippocampus. Our results suggested that hippocampal cooling with a permanently implantable system may be useful as a minimally invasive therapy for TLE, and therefore could be an effective alternative to temporal lobectomy.

**References**

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