Hyperthermic Spreading Depressions in the Immature Rat Hippocampal Slice

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Wu, Jie and Robert S. Fisher. Hyperthermic spreading depressions in the immature rat hippocampal slice. J Neurophysiol 84: 1355–1360, 2000. Febrile seizures are the most common seizure type in children (6 mo to 5 yr). The pathophysiology of febrile seizures is unknown. Current genetic studies show that some febrile seizures result from channelopathies. We have performed electrophysiological experiments in vitro hippocampal slices to test a novel hypothesis that a disordered regulation of ionic homeostasis underlies the genesis of febrile seizures. In transverse hippocampal CA1 slices from 104 rats, temperature increase from 34° to 40°C produced a series of spreading depressions (SDs), called hyperthermic SDs. The hyperthermic SDs were age-dependent, occurring in only 1/17 8–16 day-old animals, and 11/20 rats older than than 60 days. The hyperthermic SDs usually occurred on the rising phase of the temperature. The mean temperature to trigger a first hyperthermic SD was 38.8 ± 1.3°C (mean ± SD, n = 44). The hyperthermic SDs induced a reversible loss of evoked synaptic potentials and a dramatic decrease of input resistance. Neuronal and field epileptiform bursting occurred in the early phases of the hyperthermic SD. During hyperthermic SDs, pyramidal cell membrane potential depolarized by 38.3 ± 4.9 mV (n = 20), extracellular field shifted negative 18.5 ± 3.9 mV (n = 44), and extracellular K+ rose reversibly to 43.8 ± 10.9 mM (n = 6). Similar SDs could be evoked by ouabain or transient hypoxia with normal temperature. Tetrodotoxin could block initial epileptiform bursting, without blocking SDs. Hyperthermia-induced SDs should be investigated as possible contributing factors to febrile seizures.

INTRODUCTION

Febrile seizures, defined as seizures provoked by fever, without underlying encephalitis, other symptomatic etiologies, or a previous history of epilepsy, affect approximately 3–5% of the world’s children between ages 6 mo to 6 yr (Farwell 1994). The pathophysiology of febrile seizures is unknown. Familial febrile seizures have been shown to be associated with one of at least three so far elucidated gene loci: FEB1 on chromosome 8q13–21 (Wallace et al. 1996); FEB2, an autosomal dominant febrile convulsion locus FEB2 on chromosome 19p (Johnson et al. 1998); and “generalized epilepsy with febrile seizures plus” (Singh et al. 1999), which derives from mutations affecting a voltage-sensitive sodium channel (Wallace et al. 1998). Several other genes associated with familial febrile seizures are under investigation.

Since some febrile seizures result from channelopathies, it is reasonable to consider the hypothesis that febrile seizures result from a disordered regulation of ionic homeostasis, known as spreading depressions (SD). SD, originally described by Leao (1986), is a widely studied, but incompletely understood phenomenon. SDs are characterized by decreased spontaneous electrical activity, slow negative potential changes, and transmembrane ion fluxes. Ion-sensitive recordings show that SDs provoke large increases in [K+]i and decreases in [Na+]i and [Cl−]i (do Carmo and Somjen 1994). An SD initially results in neuronal hyperexcitation, followed by suppression of activity (Tepley and Wijesinghe 1996). Brain tissue from young animals is more susceptible to SD than is tissue from older animals (Luhmann and Kral 1997), roughly resembling the age-relationship of febrile seizures. Investigation of the hyperthermic in vitro rat hippocampal slice model system from rats of different ages provides an opportunity to test the hypothesis that transient epileptiform activity results from SD, such as events triggered by increased temperature.

METHODS

Experiments were performed on 104 slices from 104 rats, ages 8 days to adulthood, defined as >60 days. Data were analyzed only from the first slice studied in each rat. Rats were anesthetized with halothane and decapitated. The hippocampi rapidly were dissected and placed in iced artificial cerebrospinal fluid (CSF). Brain tissue was cooled, but not frozen, glued to a cryotome, and 500-μm transverse slices were cut through the hippocampal formations by a vibratome (Vibroslice 725 M, WPI, Sarasota, FL). Slices were allowed to incubate and recover for at least 1 h in artificial CSF comprising the following composition (in mM): 117 NaCl, 5.4 KCl, 26 NaHCO3, 1.3 MgSO4, 1.2 NaH2PO4, 2.5 CaCl2, and 10 glucose.

One slice at a time was moved from the incubation chamber to the recording chamber (FST, air-liquid interface chamber) and suspended on a nylon net at the interface. Carbogen (95% O2-5% CO2) was bubbled across the upper surface of the slice. Bath temperature was regulated by a feedback circuit, accurate to 0.5 ± 0.2°C. All temperature measurements refer to bath temperature, and not to explicit slice temperature. Baseline temperature was 34°C. After verification of evoked population spike stability for three consecutive stimuli over a time course of 30 min, bath temperature set-point was increased to 40°C and notation was made of actual temperature measured by a thermistor probe.

Extracellular field potentials were recorded with a borosilicate glass
micropipette pulled to a tip diameter of 1 μm, filled with 2 M sodium chloride and with resistance 1–10 MΩ. Intracellular recordings were performed with a pulled-glass fine-tip micropipette (<1 μm), with resistance approximately 80–100 MΩ, filled with 4 M potassium acetate. Extracellular K⁺ activity was measured with 1-μm tip microelectrodes, exposed for a few minutes to silane, and baked at 100°C for 1 h. The resin was filled approximately halfway to the shank of the electrode with potassium-sensitive resin (WPI, Sarasota, FL), and the remainder of the electrode was filled with 0.5 M KCl. The potassium-sensitive microelectrodes were calibrated before and after each experiment in Ringer solution containing, respectively, 5.4, 27, and 54 mM potassium. Voltages obtained in calibration fluid were plotted on semilog graph paper. Recorded voltages from the potassium-sensitive microelectrode during the experiment were placed on the graph and correlated to the calibration line to obtain extracellular tissue K⁺ concentration. Extracellular field potentials, recorded at a site no more than 50 μm from the potassium microelectrode, were subtracted from the potassium microelectrode via a differential amplifier, so that the recorded voltage reflected only the signal generated by the K⁺ activity. After establishing a relationship between extracellularly recorded depolarizations and rises in [K⁺]o, we omitted the cumbersome [K⁺]-sensitive microelectrodes. SDs were considered to have occurred when all of the following conditions were met: 1) at least 10 mV extracellular negativity; 2) duration of extracellular negativity at the half-height of at least 10 s; 3) loss of evoked field in CA1; and recovery of field to at least 50% of control amplitude within 30 min of cooling to baseline temperature.

Electrophysiological data were stored on a digital oscilloscope and played back on a laser printer. Slow potentials, including extracellular field and potassium changes during SD, also were recorded on a continuous rectilinear chart recorder. Chemicals used in the experiment consisted of ouabain and tetrodotoxin (Sigma, St. Louis, MO). All animal experiments were in accord with Institutional Animal Welfare Committee guidelines.

**RESULTS**

**Hyperthermia-induced SDs**

Increase of temperature from 34 to 40°C resulted in SDs. These SDs usually recurred multiple times when elevated temperature was maintained (Fig. 1A). Figure 1A shows a slow time sweep of extracellular field potential, to display multiple recurrent SD-like events. A simultaneous intracellular and extracellular recording from a slice from a 37-day-old rat after temperature was increased from 34.0 to 37.8°C over 8 min is seen in Fig. 1B. The mean temperature at which an SD was produced was 38.8 ± 1.3°C (n = 44). For 20 cells with intracellular electrodes, resting membrane potential declined from the mean baseline of −72 ± 7.7 mV by a mean depolarization of 38.3 ± 4.9 mV. The corresponding extracellular field exhibited a negative shift of 18.5 ± 3.9 mV (n = 44), with mean duration 35.6 ± 4.0 s (n = 29), measured at the halfway point to maximal negativity. Field depolarizations typically showed two components in time, with a fast exponential and a slow exponential. Population spikes evoked by stimulation of the Shaffer collateral–commissural fibers input to region CA1 provoked a robust and consistent field at baseline, with a single evoked population spike. During the peak of the SD, evoked fields were unobtainable (Fig. 2A, third column from left). Input resistance typically decreased to <10% of baseline values prior to onset of the depolarization (Fig. 2B). High temperature blocked spike frequency accommodation (data not shown), as previously described (Thompson et al. 1985).

In most slices, after production of the first SD, temperature setting was returned to 34°C. The slice returned to baseline temperature over approximately 15 min. During the cooling, membrane repolarized to the original resting membrane potential, input resistance returned to baseline values, action potential amplitude normalized, and the evoked population spike recovered to at least baseline levels.

Hyperthermic SDs occurred in an age-dependent manner. Slices from animals in three different age ranges showed SD in: 1/17 (5%) for ages 8–16 days; 44/49 (90%) for ages 17–60 days; 11/20 (55%) for ages over 60 days (Fig. 3B). However, there was no correlation between the age of the animal and the temperature at which SDs occurred (Fig. 3A).

**Extracellular K⁺ concentration**

To document the presence of SDs, potassium-sensitive microelectrodes were used to reveal the typical rise of extracellular K⁺ activity associated with SDs. Time course of the K⁺ increase to elevated temperature paralleled that of the extracellular field negativity (Fig. 4A). Simultaneous with the onset of the extracellular negativity during heating, extracellular K⁺ activity rose from mean baseline of 6.4 ± 0.2 mM (mean ± SD, n = 6) to a mean peak of 43.8 ± 10.9 mM (n = 6, P < 0.000001 by 2-tailed paired t-tests). With cooling, K⁺ returned to a mean of 6.5 ± 0.4 (n = 6, not significantly different from baseline).

We studied the time course and amplitude of SDs produced...
by block of the Na\textsuperscript{+}-K\textsuperscript{+} pump with ouabain, to document that hyperthermic depolarizations had a plausible time course for SDs in our model system. In four slices from 36, 37, 42, and 60 day-old rats, ouabain (10 m\textsuperscript{M}) produced SD-like events that were indistinguishable from hyperthermia-induced SDs (Fig. 5B), with mean depolarization of 18.3 ± 2.9 mV and half-duration of 22.8 ± 5.9 s. Extracellular depolarizations can be caused by relative hypoxia. We therefore measured field response during hypoxia induced by changing the perfusing gas from 95% O\textsubscript{2} to room air. SD-like events occurred in 6/6 (100%) of slices maintained at 34 –35°C, in animals ages 22, 27, 38, 41, 42, 60 days (Fig. 5C). The mean field depolarization for slices exposed to hypoxia was 21.2 ± 1.4 mV, and half-duration 75.3 ± 12.9 s. The amplitude of the hypoxic SD did not differ from those recorded from hyperthermic slices, although the duration of hypoxic SDs was longer (\(P > 0.05\)).

Our standard perfusate employed K\textsuperscript{+} concentration of 5.4 mM. Experiments were done in 18 slices from animals ranging in age from 16 days to adulthood perfused by 3.0 mM K\textsuperscript{+}. The hyperthermic SDs were observed in 7 (39%) of the 18 slices. Therefore, the hyperthermic SDs can occur in slices bathed with 3.0 mM K\textsuperscript{+} concentrations.

**Epileptiform activity during SDs**

At the peak of the SD, synaptic transmission and spontaneous activity ceased; however, at the starting phase of SDs, spontaneous activity increased. Figure 6A shows neuronal and field bursting at the beginning of an SD, at three different sweep speeds. Simultaneous recordings of intracellular and extracellular potentials show a clear correspondence between the two, but not every field potential corresponded with a recorded intracellular spike. Figure 6B shows emergence of spontaneous activity within a few seconds of negative field drift. As the field became approximately 5 mV negative to baseline, the spontaneous activity increased dramatically and then disappeared at 10 –15 mV field negativity. Occasional second-evoked population spikes could be observed transiently during the onset of SD, but regenerative population spikes were usually not a feature of the SDs.

To determine whether this cellular bursting was a necessary prelude to the temperature-induced SD, we added the voltage-gated sodium channel blocker, tetrodotoxin (TTX), to the perfusate. Figure 7 shows the block of the intracellular excitatory
postsynaptic potential (EPSP), action potential, and extracellular field population spike by TTX 1 μM for 20 min, with slice temperature maintained at 34.0°C. Despite the block of evoked activity, increase of temperature to 38°C still evoked a typical SD, lacking the spontaneous bursting at the early stages. Two of three slices exposed to TTX showed SDs, and one did not. The spontaneous field and cellular bursting is therefore not a necessary condition for onset of hyperthermia-induced SDs.

**DISCUSSION**

These experiments demonstrate that SD-like events occur in hippocampal slices of animals in a certain age range, when slices are heated from about 34 to 40°C. Previous literature has observed age-dependent SDs evoked by sodium acetate that first appear in baby rats at 12–15 days of age (Richter et al. 1998), corresponding to febrile seizures beginning in rats at 11 or 12 days (Baram et al. 1997). SD can occur in adult rats, but propagation velocity declines as a function of age over the span of 2.5 to 24 mo (Guedes et al. 1996). SDs are easier to produce in hippocampal slices from 18–22 day old rats than in those from 10–15 or 28–32 day old rats (Kreisman and Smith 1993). However, SDs can be made to occur as early as the second postnatal day in a rat (Psarropoulou and Avoli 1993). Age-dependent SDs can be produced by a variety of methods, including sodium acetate applied to anesthetized rat cortex (Richter et al. 1998) and by K⁺ elevation in perfusate of rat hippocampal slices (Kreisman and Smith 1993). In the present study, the hyperthermic SDs show a similar age-dependency.

Temperature changes produce a variety of effects on physiological properties of neurons. Computer models of fast excitatory synaptic transmission predict that temperature increase from 22 to 37°C would increase the EPSC rise-time four-fold and double the peak open probability (Wahl et al. 1996). However, decay is also substantially more rapid. Cooling of guinea pig hippocampal neurons increases their input resistance, enhances spike frequency adaptation, and increases the postburst after-hyperpolarization (Thompson et al. 1985). During warming of hippocampus a few degrees (Anderson and Moser 1995; Eckerman et al. 1990), action potentials have shorter rise and decay times; action potential amplitude becomes slightly smaller; axonal conduction velocity increases, and transmitter release becomes faster and more synchronized. Submerged slices heated from 29 to 37°C show increase of the extracellularly recorded EPSP, but at the same time loss of the population spike, consistent with membrane hyperpolarization (Schiff and Somjen 1985). Immature brain reacts differently than does mature brain to temperature increase (Shen and Schwartzkroin 1988). The multiplicity of effects of temperature on membrane and synaptic properties make prediction of net effects on excitability difficult.

The temperature dependence of cortical KCl-induced SDs...
was studied in anesthetized rat cortex (Takaoka et al. 1996). The electrical threshold to initiate SDs and the amplitude of SDs were not changed by varying temperature from 33 to 40°C, but increased temperature did increase the propagation rate of SDs. Reduction of temperature of hippocampal slices from 36 to 31°C eliminated hypoxic SDs (Taylor and Weber 1993). GABA function may be impaired during SDs (Janigro and Schwartzkroin 1987) and for at least a week after a febrile seizure (Chen et al. 1999). In neocortical slices from adult rats, SDs reduced paired pulse inhibition by 10%, with no change in excitatory transmission (Kruger et al. 1996). In our experiments, we rarely saw regenerative evoked population spikes during SDs, suggesting that GABAergic systems were still in evidence. We argue that SDs, and not simple failures of GABAergic systems, lead to hyperthermic epileptiform activity in the hippocampal slice model system.

Our results do not specify the mechanism by which heating brain slices leads to SD. However, studies of SD in other model systems suggest that the increased energy and oxygen requirements associated with higher temperatures may be a factor. In chick retina, SDs result in a series of changes in pH, ATP, ADP, and phosphocreatine levels. During SDs, ATP and AMP remain constant, but ADP increases and phosphocreatine decreases by 38% (Lauritzen et al. 1990). High-energy phosphate stores are depleted at the advancing edge of an SD (Gault et al. 1994), and anaerobic glycolysis may become dominant (Kocher 1990). More than 60% of brain energy consumption is consumed by active transport processes (Mayevsky et al. 1998), and the sodium-potassium ATP-dependent pump is one of the most important of these processes. The sodium-potassium pump normally restores intracellular-extracellular ion balance after periods of neuron excitation. SDs can be initiated under suitable conditions (Haglund and Schwartzkroin 1990) by blocking the sodium-potassium pump, for example, with ouabain (current results and Basarsky et al. 1998). Pump failure at high temperature could underlie hyperthermic SDs.

Relevance of our in vitro findings to the clinical condition of febrile seizures presently is unknown. Heating animals by water baths or hot air can lead to febrile seizures with behavioral and EEG characteristics resembling clinical febrile seizures, and with long-lasting effects on brain excitability (Chen et al. 1999). Local application of heat to intact skull can produce SDs in rats (Rangel and Welsh 1999). No studies yet address the presence or absence of SDs in the brain of system-
ically hyperthermic animals. However, the time course of epileptiform activity in our in vitro model system is much briefer than that of a clinical febrile seizure.

During the initial phase of a SD, we observed epileptiform bursting of action potentials and field potentials. This is most likely explained by neuron membrane depolarization from increased extracellular K⁺ activity that closely corresponded in time course to field potential changes. In our model system, SDs did not depend on such neuronal bursting, since TTX could eliminate bursting activity without always blocking SDs. The SD therefore cannot be considered an epiphenomenon of epileptiform activity. Our results provide grounds to consider whether hyperthermic SDs might be one factor in the genesis of febrile seizures.

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