Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/brainres


Research Report

# Hypothermia did not prevent epilepsy following experimental status epilepticus

Mirja Steinbrenner[a,1], Alexander B. Kowski[a,1], Friedhelm C. Schmitt[b], Martin Holtkamp[a,*]

[a]Epilepsy-Center Berlin-Brandenburg, Department of Neurology, Charité - Universitätsmedizin Berlin, Charitéplatz 1, 10117 Berlin, Germany

[b]Department of Neurology, Universitätsklinik Magdeburg, Leipziger Str. 44, 39120 Magdeburg, Germany

ARTICLE INFO

*Article history:*
Accepted 12 May 2014
Available online 20 May 2014

*Keywords:*
Brain insult
Cooling
Excitation
Inhibition
Spontaneous seizure

ABSTRACT

In epilepsy research, one of the major challenges is to prevent or at least mitigate development of epilepsy following acquired brain insult by early therapeutic interventions. So far, all pharmacological antiepileptogenic treatment approaches were largely unsuccessful in clinical trials and in experimental animal studies. In a well-established rat model of chronic epilepsy following self-sustaining status epilepticus (SSSE), we assessed the antiepileptogenic properties of 3-h-cooling induced directly after the end of SSSE. Occurrence of spontaneous seizures and seizure severity up to 8 weeks after SSSE were compared with normothermic SSSE controls. Furthermore, electrophysiological parameters assessing inhibition and excitation in the dentate gyrus were assessed at multiple time points. Post SSSE hypothermia did not prevent the occurrence of seizures in any animal. Eight weeks after SSSE, Racine motor seizures trended to be less severe following cooling ($4.0 \pm 0.6$) compared with normothermic controls ($4.8 \pm 0.2$) but the difference was not significant when testing for multiple comparisons. Early loss of inhibition that is typically seen following SSSE was somewhat attenuated in cooled animals 3 h after SSSE as expressed by smaller paired-pulse ratios (PPR; $0.16 \pm 0.21$) compared with normothermic controls ($0.54 \pm 0.21$) but difference was not significant either. Latency between stimulus artefact and excitatory post-synaptic potential 3 h after SSSE, reciprocally reflecting neuronal excitation, was higher in animals that underwent hypothermia ($8.29 \pm 2.45$ ms) compared with controls ($4.82 \pm 0.66$ ms), difference was not significant after correction for multiple comparisons. In summary, the current experiments were not able to demonstrate prevention or mitigation of epileptogenesis with immediate short-term cooling following SSSE.



*Corresponding author. Fax: +49 30 450 560 932.
*E-mail addresses:* mirja.steinbrenner@charite.de (M. Steinbrenner), alexander.kowski@charite.de (A.B. Kowski), fc.schmitt@med.ovgu.de (F.C. Schmitt), martin.holtkamp@charite.de (M. Holtkamp).

[1]MS and ABK contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.brainres.2014.05.018
0006-8993/

## 1. Introduction

Antiepileptic treatment commonly refers to suppression of unprovoked epileptic seizures in established epilepsy that in fact represents an antiictal treatment strategy. In patients with acquired brain insults such as traumatic brain injury or status epilepticus, which imply a high risk of subsequent epilepsy, early therapeutic interventions aim to completely prevent or at least mitigate the development of epilepsy representing an antiepileptogenic approach (Pitkanen and Lukasiuk, 2011).

Antiepileptic drugs that are successful antiictally and a plethora of other pharmacological agents with various mechanisms of action have been assessed regarding their antiepileptogenic properties in a few clinical trials and manifoldly in animal models of epileptogenesis induced by diverse brain insults. In summary, none of these studies demonstrated an antiepileptogenic effect (Holtkamp and Meierkord, 2007; Kobow et al., 2012; Loscher and Brandt, 2010).

Hypothermia, a non-pharmological treatment approach, is well-known to reduce cortical excitability. Thus, it has been applied in patients to suppress circumscribed intraoperative spiking in epilepsy surgery (Karkar et al., 2002) and to contribute to management of refractory status epilepticus (Guilliams et al., 2013; Orlowski et al., 1984). In an experimental model of electrically induced self-sustaining status epilepticus (SSSE), our group has demonstrated strong anticonvulsant effects of moderate (30 °C) and deep (20 °C) hypothermia eventually terminating continuous seizure activity (Kowski et al., 2012; Schmitt et al., 2006). In animal models of fluid percussion injury, seizure susceptibility was attenuated by early hypothermia (Atkins et al., 2010) and epileptogenesis was largely prevented by mildly lowering perilesional temperature by 2 °C for 5 weeks (D'Ambrosio et al., 2013). In the latter study, interventional hypothermia was initiated 3 d after traumatic brain injury.

Relevant pathophysiological changes promoting epileptogenesis such as accumulation of *N*-methyl-D-aspartate (NMDA) receptors (McNamara et al., 2006) have been described to occur in the first hour after experimental status epilepticus (Naylor et al., 2013). Due to some antiglutamatergic effects of cooling (Van Hemelrijck et al., 2003; Winfree et al., 1996), we hypothesized that induction of hypothermia for 3 h directly after termination of SSSE has antiepileptogenic properties. At various time points up to 8 weeks following SSSE, we assessed occurrence and severity of spontaneous seizures by intermittent video recording and dentate gyrus (DG) inhibition and excitation by in vivo electrophysiological measurements (for overview, see Fig. 1).

## 2. Results

### 2.1. *Self-sustaining status epilepticus*

In 23 animals, the perforant path was stimulated electrically for 2 h resulting in SSSE in 21 animals. The remainder did not fulfill our inclusion criteria as regularly occurring spontaneous discharges ($\geq$1 Hz) at the end of stimulation were lacking. These 21 animals maintained SSSE for the next 3 h. Two animals died directly after pentobarbital-induced termination of SSSE. Thus, a total of 19 animals were eligible for post-SSSE hypothermia or normothermia and for subsequent video recording and electrophysiological studies. In the electrode control group, nine animals were injected intraperitoneally (i.p.) pentobarbital at an index time point without prior induction of SSSE.

### 2.2. *Induction of hypothermia*

Five minutes after i.p. administration of 30 mg/kg pentobarbital, SSSE animals either underwent cooling down to 25 °C ($n$=9) or were maintained at 37 °C core temperature (normothermic controls, $n$=10) for 3 h. Electrode controls ($n$=9) were kept at 37 °C for 5 h after administration of pentobarbital. In cooled animals, the target temperature was reached after 40±13 min. At the end of induced hypothermia, this group of rats was rewarmed to 37 °C over another 2 h. Fig. 2 illustrates the course of body temperature in both groups of animals following SSSE.

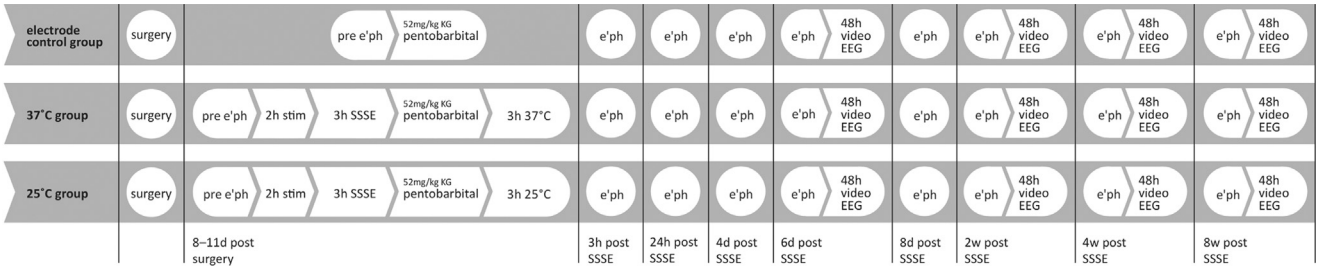


**Fig. 1 – Study overview. Animals of all three groups underwent implantation of electrodes into the right perforant path and the ipsilateral dentate gyrus. Two groups underwent electrical induction of self-sustaining status epilepticus (SSSE) that after 3 h was terminated with pentobarbital, while animals of the electrode control group remained unstimulated but were also administered pentobarbital. After the end of SSSE, one of the two groups underwent immediate cooling to 25 °C body temperature that was maintained for 3 h. The other post-SSSE group and the electrode controls were kept at 37 °C. Animals of all groups were video-monitored for periods of 48 h to detect behavioral seizures 1, 2, 4 and 8 weeks after SSSE. All animals underwent electrophysiological (e'ph) measurements to assess dentate gyrus inhibition and excitation 3 h, 24 h, 4 d, 6 d, 8 d, 2 weeks, 4 weeks and 8 weeks after SSSE.**