

Epileptiform Activity During Rewarming from Moderate Cerebral Hypothermia in the Near-Term Fetal Sheep

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ABSTRACT

Moderate hypothermia is consistently neuroprotective after hypoxic-ischemic insults and is the subject of ongoing clinical trials. In pilot studies, we observed rebound seizure activity in one infant during rewarming from a 72-h period of hypothermia. We therefore quantified the development of EEG-defined seizures during rewarming in an experimental paradigm of delayed cooling for cerebral ischemia. Moderate cerebral hypothermia ($n = 9$) or sham cooling ($n = 13$) was initiated 5.5 h after reperfusion from a 30-min period of bilateral carotid occlusion in near-term fetal sheep and continued for 72 h after the insult. During spontaneous rewarming, fetal extradural temperature rose from $32.5 \pm 0.6^\circ\text{C}$ to control levels ($39.4 \pm 0.1^\circ\text{C}$) in 47 ± 6 min. Carotid blood flow and mean arterial blood pressure increased transiently during rewarming. The cooling group showed a significant increase in electrical seizure events 2, 3, and 5 h after rewarming, maximal at 2 h (2.9 ± 1.2 versus 0.5 ± 0.5 events/h; $p < 0.05$). From 6 h after rewarming, there was no

significant difference between the groups. Individual seizures were typically short (28.8 ± 5.8 s versus 29.0 ± 6.8 s in sham cooled; NS), and of modest amplitude (35.9 ± 2.8 versus 38.8 ± 3.4 μV ; NS). Neuronal loss in the parasagittal cortex was significantly reduced in the cooled group ($51 \pm 9\%$ versus $91 \pm 5\%$; $p < 0.002$) and was not correlated with rebound epileptiform activity. In conclusion, rapid rewarming after a prolonged interval of therapeutic hypothermia can be associated with a transient increase in epileptiform events but does not seem to have significant adverse implications for neural outcome. (*Pediatr Res* 57: 342–346, 2005)

Abbreviations

CaBF, carotid blood flow
EAA, excitatory amino acid
HIE, hypoxic-ischemic encephalopathy

There is now consistent experimental evidence that a sufficient interval of moderate cerebral hypothermia started shortly after severe perinatal hypoxia-ischemia can reduce secondary neuronal injury and improve functional outcomes (1). Although only pilot data are available in newborn infants (2), early induction of moderate hypothermia in adult patients after cardiac arrest improves neurologic recovery (3,4). Rewarming after cooling treatment for severe hypoxic-ischemic encephalopathy (HIE), however, is potentially a more complex process

than the original induction of cooling: first because it might lead to destabilizing cardiovascular changes such as a loss of peripheral tone with an increased requirement for cardiac work (5) and second because damaging processes such as release of excitatory amino acid neurotransmitters that have been suppressed by hypothermia could become active again (6,7).

In view of such considerations, clinical studies of therapeutic hypothermia typically have rewarmed infants at no more than $0.5^\circ\text{C}/\text{h}$ (8–10); however, there are few systematic data. During our early pilot studies of head-cooling with mild systemic hypothermia for neonatal HIE, we observed a case of an infant who exhibited transient clinical seizures during rewarming. To examine the hypothesis that rewarming from prolonged moderate hypothermia may be associated with rebound seizure activity, we quantified changes in numbers of epileptiform events and in their duration and intensity after rapid rewarming from a prolonged interval of *in utero* moderate hypothermia for

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cerebral ischemia in near-term fetal sheep. The sheep is a highly precocial species; thus, in terms of cerebral maturity and myelination, this gestational age is comparable to the human brain at term (11). We have previously reported the electrophysiologic, histologic, and blood changes in a subset of this group (12).

METHODS

In this study, we used the approach of selectively cooling the head to minimize the systemic complications associated with whole-body hypothermia (13). A chronically instrumented fetal preparation was used to test the effect of postsult cooling; this is clearly a compromise as fetal and neonatal metabolism differ in several aspects. However, this approach provides a primary insult that occurs under fetal conditions, ensures stable control temperatures, and avoids anesthesia that might interact with hypothermia (14).

Surgical procedures. The experimental studies were approved by the Animal Ethics Committee of the University of Auckland. Romney/Suffolk fetal sheep from 117 to 124 d of gestation were operated on under 2% halothane/oxygen anesthesia as previously reported (12,15). Polyvinyl catheters were inserted into both fetal brachial arteries and the amniotic sac. The vertebral-occipital anastomoses were ligated bilaterally to restrict vertebral blood supply to the carotid arteries. A double-ballooned inflatable occluder cuff was placed around each carotid artery along with a 3S-mm ultrasonic flow probe (Transonic Systems, Ithaca, NY) just proximal to the cuff. Two pairs of EEG electrodes (AS633-5SSF; Cooner Wire Co., Chatsworth, CA) were placed on the dura over the parasagittal parietal cortex (5 and 15 mm anterior and 10 mm lateral to the bregma), with a reference electrode sewn over the occiput. For measuring cortical impedance, a third pair of electrodes (Cooner Wire AS633-3SSF) was placed over the dura, 5 mm lateral to the EEG electrodes. A thermistor (Incu-Temp-1; Mallinckrodt Medical, St. Louis, MO) was placed over the parasagittal dura 20 mm anterior to the bregma; the burr holes were sealed, and the skin over the fetal skull was closed using cyanoacrylate glue.

A cooling coil, made from silicone tubing (Degania Silicone, Degania Bet, Israel), was attached over the dorsal surface of the scalp and extended over the lateral surface of the cranium down to the level of the external auditory meatus. A second thermistor (to measure fetal core body temperature) was placed in the fetal esophagus at the level of the right atrium. The fetus then was returned to the uterus, and all leads and catheters were exteriorized to the maternal flank. A polyvinyl catheter was placed in the maternal tarsal vein. After the operation, the ewes were housed in metabolic cages at constant temperature ($16 \pm 1^\circ\text{C}$) and humidity ($50 \pm 10\%$), in a 12-h light/dark cycle, with free access to food and water. Gentamicin, 80 mg i.v., was administered daily to the ewe for 5 d.

Recordings. Fetal arterial blood pressure, corrected for amniotic fluid pressure (Novatrans II, MX860; Medex, Hilliard, OH), carotid arterial blood flow (T208 Ultrasonic Flowmeter; Transonic Systems), fetal extradural temperature, esophageal temperature, and fetal parietal EEG and impedance were recorded continuously. Recordings started at least 12 h before the experiment and continued for 120 h afterward. Signals were averaged at 1-min intervals and stored to disk by custom software (Labview for Windows; National Instruments Ltd, Austin, TX), running on an IBM compatible computer. The EEG signal was low-pass filtered at 30 Hz, and then the intensity spectrum and impedance signal were extracted. The raw EEG signal was recorded for off-line detection of seizure events.

Experimental procedures. At least 3 d after surgery, reversible cerebral ischemia was induced by inflating the carotid cuffs bilaterally with sterile saline for 30 min. Successful occlusion was confirmed by the onset of an isoelectric EEG signal within 30 s of inflation (16).

Fetuses were randomized to either cooling or sham cooling starting at 6 h after ischemia (*i.e.* 5.5 h after reperfusion) and continued until 72 h. Cooling was performed by circulating cold water through the cooling coil; the initial water temperature was 6°C and then adjusted as necessary in the first 4 h to obtain an extradural temperature between 30 and 33°C . In sham-cooled fetuses, the water was not circulated; thus, the cooling coil and its contents will have remained in equilibrium with fetal temperature. Fetuses were allowed to rewarm spontaneously after cooling was stopped. The fetuses were monitored for 48 h after the end of cooling, and then the ewe and the fetus were killed by an overdose of sodium pentobarbitone (9 g i.v. to the ewe; Pentobarb 300; Chemstock Int., Christchurch, New Zealand).

The fetal brain was perfusion-fixed *in situ* with 10% phosphate-buffered formalin. Predefined sections were selected according to the stereotaxic atlas for the immature fetal sheep. Neuronal loss was scored by light microscopy on

8- μm -thick coronal sections stained with thionin and acid fuchsin by an assessor who was masked to the treatment group (12,15). The proportion of neurons showing ischemic cell change in 48 preassigned areas in the parasagittal cortex was scored on a six-point scale: 0 = no dead neurons; 5 = >0 –10%; 30 = >10 –50%; 70 = >50 –90%; 95 = 90– $<100\%$; 100 = 100% dead neurons. Average scores were calculated for each fetus.

Data analysis and statistics. Carotid blood flow (CaBF) was calculated as the sum of the left and right measurements. CaBF is an index to cerebral blood flow, with a strong correlation with direct microsphere measurements (17,18). Numbers of electrical seizure events were defined on EEG recordings from 6 h before rewarming to 24 h afterward in 13 sham-cooled and nine cooled fetuses. Seizure events were defined on raw EEG recordings by the presence of stereotypic evolving waveforms showing stereotypic increase and decrease in activity that lasted for 10 s or more and contained at least one peak of 20 μV or greater, followed by a depression in EEG activity (19). When the raw EEG recording was not available (one of 13 sham-cooled animals and two of nine cooled animals), an electrical seizure event was defined on the 1-min EEG recording as a peak with a maximum of at least 10 μV followed by a decrease in activity with a minimum of 10 μV or less. The duration of seizure events was calculated on raw EEG recordings only.

The effect of rewarming on time-series analysis of physiologic data was performed off-line on the recorded signals, using hourly averages, by ANOVA (SPSS; SPSS Inc., Chicago, IL); time was treated as a repeated measure. The Mann-Whitney *U* test was used to examine differences between groups in the frequency of electrical seizure events and histologic scores. Fisher exact test was used to examine changes in proportions. Regression analysis was used to examine the relationship between the increase in numbers of events in the 6-h period after rewarming to neuronal cell loss in the parasagittal region of the cortex. Data are mean \pm SEM.

RESULTS

In the cooled group, fetal extradural temperature rose from $32.5 \pm 0.6^\circ\text{C}$ in the final 6 h of cooling to control levels ($39.4 \pm 0.1^\circ\text{C}$) by 47 ± 6 min after the start of rewarming ($p < 0.001$; Fig. 1). Subsequently, there was a trivial level of overshoot of both extradural and esophageal temperatures compared with sham-cooled fetuses (to $39.7 \pm 0.1^\circ\text{C}$ versus 39.5 ± 0.1 ; NS). There was a small increase in CaBF in the first hour after the start of rewarming compared with sham controls (108.6 ± 7.9 versus 90.2 ± 10.3 mL/min; $p < 0.05$; Fig. 1). Mean arterial blood pressure also showed a transient significant increase at 2 and 3 h after rewarming ($p < 0.05$). Although there was a small apparent rise in fetal heart rate in the first hour, this was not significant compared with sham controls (Fig. 1).

Rebound electrical seizure events were observed in five of nine cooled and only one of 13 sham-cooled animals ($p < 0.05$, Fisher exact) during the first 6 h after rewarming (Fig. 2). The cooling group showed a significant increase in electrical seizure events 2, 3, and 5 h after rewarming, maximal at 2 h (2.9 ± 1.2 versus 0.5 ± 0.5 events/h; $p < 0.05$). From 6 h after rewarming, there was no significant difference between the groups. The duration of rebound seizure events was short, with an average of 28.8 ± 5.8 s in the cooled and 28.9 ± 6.8 s in the sham-cooled groups. Similarly, the amplitude of seizure events in the 6 h after rewarming was moderate and not different between the groups (35.9 ± 2.8 versus 38.8 ± 3.4 μV). Parasagittal neuronal cell loss was less in cooled fetuses than in controls ($51 \pm 9\%$ versus $91 \pm 5\%$; $p < 0.002$), and there was no relationship between rebound seizure events and neuronal cell loss (Fig. 3).

DISCUSSION

This study demonstrates that 3 d after a severe ischemic insult that was treated with moderate cerebral hypothermia,

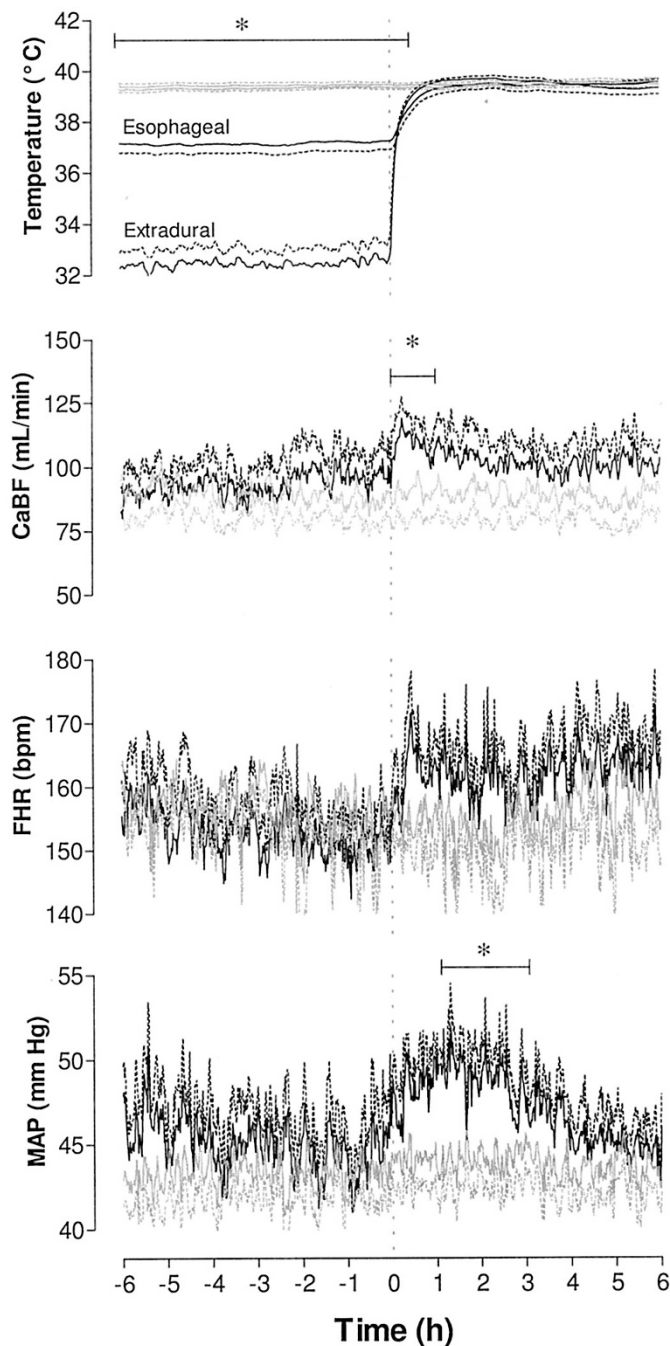


Figure 1. Time sequence of changes in extradural and esophageal temperatures, carotid blood flow (CaBF), fetal heart rate (FHR; beats per minute), and mean arterial blood pressure (MAP) in sham-cooled (grey solid line; $n = 13$) and cooled (black solid line; $n = 9$) fetuses. The dashed lines indicate the respective SEMs. After the onset of rewarming at time 0, shown by the vertical dotted lines, extradural temperature returned to control levels in 47 ± 6 min. Data are mean \pm SEM; $*p < 0.05$.

rapid rewarming was associated with a significant but transient increase in EEG-defined seizure events and only mild systemic changes such as a transient increase in blood pressure and CaBF. Seizures are common in both infants with HIE (2,9) and experimental models of neonatal HIE (12,15). Although they typically are most severe in the first 24 h and resolve with time (15), some seizures may continue for many days. Thus, potentially, seizures that occur by coincidence during rewarming

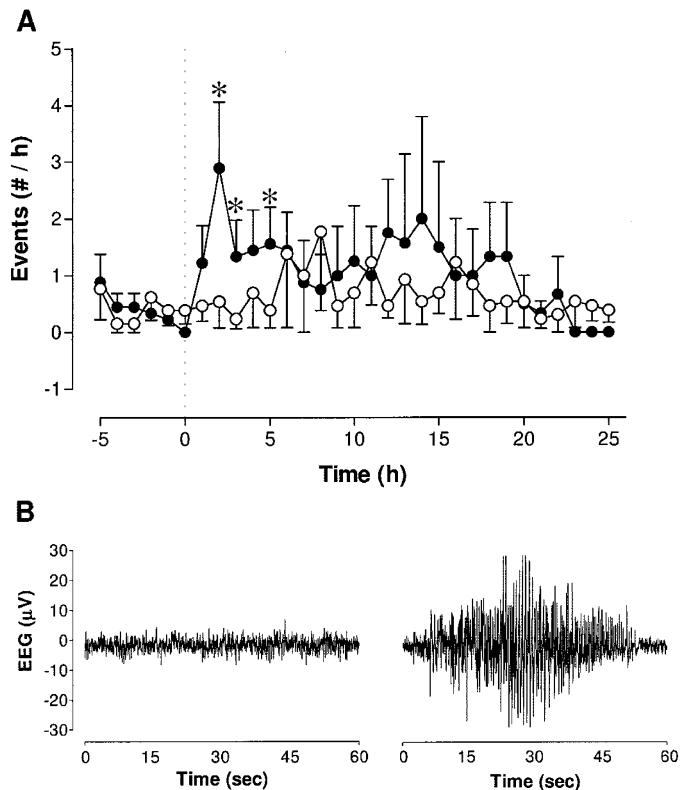


Figure 2. (A) Time sequence of changes in frequency of EEG-defined seizure events per hour. There was a significant increase in the frequency of events between 2 and 5 h after the start of rewarming in cooled fetuses (\bullet ; $n = 9$) compared with sham-cooled fetuses (\circ ; $n = 13$), which rapidly resolved to control values from 6 h. $*p < 0.05$ (Mann Whitney U test). (B) Examples of 1-min periods of raw EEG recordings from a cooled fetus. The left panel shows suppressed EEG, before the start of rewarming. The right panel shows a seizure event taken from the second hour after the start of rewarming.

could be confounded with “rebound” activity. We reported previously that seizure activity tends to be reduced during cooling (12). Combined with the observation of a transient increase in clinical seizures even after the much slower clinical rate of rewarming (over 3 h versus 45 min) from head cooling with mild systemic hypothermia in an infant with evidence of HIE, the present data strongly suggest that rebound seizure activity can occur, at least occasionally. Nevertheless, it is important to note that the events were of similar, short duration as those that occurred in sham-cooled fetuses and that from 6 h after the start of rewarming onward, the frequency of events was not significantly different from controls. Critically, despite the presence of this rebound activity, significant neuroprotection was seen in the parasagittal region of the cortex, similar to our previous report in a subset of this cohort (12).

Although there are few data relating to the impact of rewarming after a period of prolonged cooling for preceding brain injury, there are suggestive data from studies of acute cooling. In adult dogs, rewarming from hypothermic cardiopulmonary bypass is associated with progressive recovery of normal continuous EEG activity (20); however, in this setting, there should seldom be underlying brain injury. In adult rats, although rapid rewarming over 30 min from moderate hypothermia was not associated with persistent biochemical or

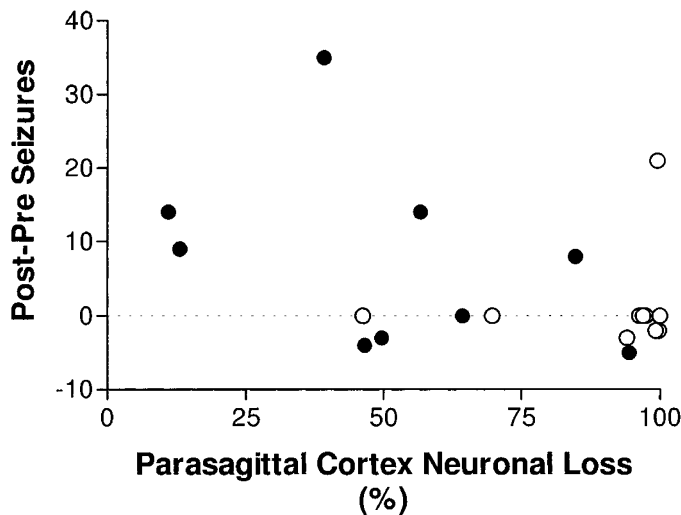


Figure 3. Relationship between rebound seizure events (total number of events in the 6-h interval after rewarming – total number of events in the 6 h before rewarming) and neuronal cell loss in the parasagittal cortex in sham-cooled (○; $n = 13$) and cooled (●; $n = 9$) fetuses. There was no significant relationship between the number of rebound seizure events and the severity of neuronal cell loss after 5 d of recovery from ischemia ($r = -0.33$, $p = 0.29$).

behavioral changes (21), there were greater early changes in cardiac output and heart rate, in a similar pattern to the present study, compared with slower rewarming over 2 h. Similarly, others have reported that rapid rewarming over 30 min can cause transient uncoupling of cerebral circulation and metabolism, with a transient increase in extracellular glutamate and lactate (22). Of greater concern, very rapid rewarming over 15 min after 1 h of hypothermia in adult rats can exacerbate traumatic axonal injury and impair cerebrovascular responsiveness, compared with slow rewarming over 90 min (23,24). Although these studies used very short periods of hypothermia combined with faster rewarming than the mean of 47 min in our experimental study, they support the concept that very rapid rewarming can have undesirable physiologic and neural effects.

Several lines of evidence indicate that changes in brain temperature affect the generation of epileptiform activity. Hypothermia has been shown to directly reduce the rate of excitatory amino acid (EAA) neurotransmitter release during ischemic depolarization (7,25), whereas posthypoxic cooling reduces EAA accumulation (26). Furthermore, hypothermia has potent postsynaptic effects on injured neurons as shown by the reduction in neuronal degeneration seen *in vitro* when hypothermia is started immediately after brief exposure to high concentrations of glutamate (27). Consistent with this, in the adult rat, hypothermia has been shown to increase the threshold for both flurothyl-induced status epilepticus and electroshock seizures (28,29). Similarly, hypothermia after global hypoxia-ischemia in the piglet was associated with reduced numbers of seizures (30). Thus, an increase in neuronal excitability during rewarming, coupled with loss of inhibition of EAA release, may have contributed to the transient increase in electrical events in the present study. Conversely, because hyperthermia can reduce the threshold for seizures (29), an overshoot in-

crease in brain temperature after rewarming could be a possible contributor to rebound seizures. However, although there was a small increase in extradural temperatures over control values in the present study, it was not significant.

An alternative explanation is that the rebound seizures were a reflection of secondary deterioration that had been only temporarily interrupted or masked by hypothermia (31). This is unlikely to be a factor in the present study because there was no relationship between the occurrence or severity of rebound seizures in the first 6 h and the severity of neuronal loss in the underlying cortex 48 h after rewarming. Indeed, overall, there was significantly less damage in the hypothermia group, and the two cooled fetuses that showed the most rebound activity were among the least damaged. This indicates that one possible factor may simply be the presence of more surviving neurons that are able to exhibit epileptiform events. Evolving neural effects may extend for longer than the 5-d recovery from ischemia in the present study (32); thus, further longer term outcome studies would be necessary to confirm the present findings. Nevertheless, in adult rodents, persistent neuronal protection with hypothermia has been found 2–6 mo after ischemia (33,34).

CONCLUSION

In conclusion, the present data demonstrate that a transient increase in electrical seizure events is not uncommon during very rapid rewarming in the experimental paradigm. From the clinical perspective, it is important to advise parents that this may occur; however, such activity does not seem to have any significant adverse implications for neurologic recovery or physiologic adaptation. Further studies are needed to test whether slower rewarming may reduce the incidence of rebound seizures.

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