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Twenty-Four Hours of Mild Hypothermia in Unsedated Newborn Pigs Starting after a Severe Global Hypoxic-Ischemic Insult Is Not Neuroprotective

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ABSTRACT

Three to 12 h of mild hypothermia (HT) starting after hypoxia-ischemia is neuroprotective in piglets that are anesthetized during HT. Newborn infants suffering from neonatal encephalopathy often ventilate spontaneously and are not necessarily sedated. We aimed to test whether mild posthypoxic HT lasting 24 h was neuroprotective if the animals were not sedated. Thirty-nine piglets (median weight 1.6 kg, range 0.8–2.2 kg; median age 24 h, range 7–48 h) were anesthetized and ventilated and subjected to a 45-min hypoxic (Fio_2 ~ 6%) global insult (n = 36) or sham hypoxia (n = 3). On reoxygenation, 18 were maintained normothermic (NT, 39.0°C) for 72 h, and 21 were cooled from 39 (NT) to 35°C (HT) for the first 24 h before NT was resumed (18 experimental, three sham hypoxia). Cardiovascular parameters and intermittent EEG were documented throughout. The brain was perfusion fixed for neuropathology and five main areas examined using light microscopy. The insult severity (duration in minutes of EEG amplitude < 7μV) was similar in the NT and HT groups, mean ± SD (28 ± 7.2 *versus* 27 ± 8.6 min), as was the mean Fio_2 (5.9 ± 0.7 *versus* 5.8 ± 0.8%) during the insult. Six NT and seven HT piglets developed posthypoxic seizures that lasted 29 and 30% of the time, respectively. The distribution and degree of injury (0.0–4.0, normal-maximal damage) within the brain (hippocampus, cortex/white matter, cerebellum, basal ganglia, thalamus) were similar in the NT and HT groups (overall score, mean ± SD, 2.3 ± 1.5 *versus* 2.4 ± 1.3) as was the EEG background amplitude at 3 h (13 ± 3.5 *versus* 10 ± 3.3 μV). The HT animals shivered and were more active. The sham control group (n = 3) shivered but had normal physiology and neuropathology. Plasma cortisol was significantly higher in the HT group during the HT period, 766 ± 277 *versus* 244 ± 144 μM at 24 h. Mild postinsult HT for 24 h was not neuroprotective in unsedated piglets and did not reduce the number of animals that developed posthypoxic seizures. Cortisol reached 3 times the NT value at the end of HT. We speculate that the stress of shivering and feeling cold interfered with the previously shown neuroprotective effect of HT. Research on the appropriateness of sedation during clinical HT is urgent. **(*Pediatr Res* 50: 405–411, 2001)**

Abbreviations

LA EEG, low-amplitude EEG
Fio_2, fraction of inspiratory oxygen
$Paco_2$, arterial P_{CO_2}
Pao_2, arterial P_{O_2}
apH, arterial pH
SaO_2, arterial oxygen saturation
$TcSaO_2$, transcutaneous arterial oxygen saturation
MABP, mean arterial blood pressure
HI, hypoxia-ischemia
HT, hypothermia
NT, normothermia
T_{rectal}, rectal temperature

Many studies on newborn animal models show that mild HT, which starts after an HI insult, is neuroprotective (1–5). The duration of HT in these studies varies from 3 to 72 h, and the reduction in body temperature varies between 3.5 and 6°C. Some studies, however, show only a minor beneficial effect (6), or the neuroprotection is temporary and not maintained (7).

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Most models of neonatal HI involve surgery for cerebral vessel occlusion and differ from clinical asphyxia in that only the brain and not the rest of the body is subject to the insult. We have developed a noninvasive global hypoxia model using the newborn piglet that develops clinical signs, particularly seizures, EEG abnormalities, and neuropathologic changes similar to those seen in infants suffering from neonatal encephalopathy (8).

Unlike adults and children after brain trauma (9, 10), newborn human infants with neonatal encephalopathy are not routinely anesthetized. Depending on local practice, the infants may be sedated during mechanical ventilation and intensive care and may receive anticonvulsive treatment if they develop clinical seizures (11).

The aim of this study was to examine whether mild HT (a reduction in body temperature of 4°C) for 24 h after global HI in newborn piglets reduces *1*) the extent of neuropathologic damage at 72-h survival and/or *2*) the occurrence of posthypoxic seizures.

METHODS

The present study was approved by the Norwegian Experimental Animal Board. Forty-four healthy Landrace newborn pigs of either sex were removed from the sow <3 h before the experiment started. Their median age was 24 h (range 7–48 h), median weight 1600 g (range 820–2230 g), median litter size 10.5 (range 4–17), and the median number of still births per litter 0 (range 0–3). During and after transport, the piglets were kept in a warm quiet environment to minimize stress. They were bottle-fed pig formula *ad libitum* on arrival.

Two piglets were used in pilot experiments to investigate whether pethidine 10 mg/kg i.v. reduced shivering during hypothermia (T_{rectal}, 35°C) (12). Visible shivering was only mildly reduced, whereas spontaneous respiration was suppressed, arterial P_{CO_2} rose, and SaO_2 fell. We, therefore, accepted spontaneous shivering in the cooled animals in our experimental design (13, 14) so as not to impair spontaneous breathing. Three piglets were used as sham controls for the examination of the effect of unsedated HT without hypoxia on brain pathology. The experimental group comprised 39 piglets of which three died and were replaced. One animal had a massive pulmonary hemorrhage as well as profuse bleeding during preparation, and another had a sudden cardiac arrest before the insult. A third pig (6 h into HT) had a cardiac arrest as a result of a drug error.

Details of the methods and monitoring used have been reported previously (8). Briefly, animals were N_2O/halothane anesthetized, intubated, and ventilated with 66% N_2O, 33 to 34% O_2, and 0.6–0.8% halothane. During baseline conditions, the ventilation was adjusted to keep end-tidal CO_2 at 5.0 to 6.0 kPa and $TcSaO_2$ at 95 to 98%. Deep rectal (at 6 cm) temperature was kept stable and normal for pigs at 39°C (range 38.4–39.5°C) (15) and used as core temperature (16).

The umbilical vein and artery were catheterized for fluid infusion, blood sampling, and continuous arterial blood pressure monitoring. Arterial blood gases, glucose, and samples for hematology and biochemistry were sampled twice before, twice during, and at 1, 3, 6, 12, 24, 30, 48, and 72 h after the 45-min hypoxic insult. Baseline observations lasted a minimum of 60 min of stable blood gases, blood pressure, blood glucose, and normal temperature.

Two-channel EEG was recorded from skin electrodes over each hemisphere with interelectrode distances of 3 cm, the placement corresponding to F1-P3 and F2-P4. The raw EEG signal was continuously recorded on tape (Oxford Medilog 9000, Oxford Medical, Abingdon, UK) before, during, and for the first 6 h after the insult and then intermittently throughout (8).

N_2O was turned off 30 min before the insult, and anesthesia was maintained during the insult with 0.8% halothane, which is one minimum alveolar concentration (1 MAC) for newborn pigs at 39°C (17) and has a cardiodepressant effect. Hypoxia was induced by reducing Fio_2 to ~6%. Fio_2 was then adjusted to the maximal level that gave LA EEG < 7 μV while at the same time avoiding severe hypotension (MABP < 30 mm Hg) and bradycardia [heart rate (HR) < 100 beats/min]. Severe bradycardia not responding to a transient increase in Fio_2 was treated with chest compression in one NT and two HT animals for 1–3 min. Hypoxia was maintained for 45 min followed by reoxygenation. The Fio_2 was adjusted to give $TcSaO_2$ 95 to 98%. Anesthesia was stopped on reoxygenation. This is a change from previous studies with this model in which anesthesia was maintained for the first 6 h after the insult, *i.e.* during the period of reoxygenation followed by HT or NT (6, 18, 19).

One-third correction of the base deficit by i.v. buffer [trometamol:tris(hydroxymethyl)aminomethane (THAM)], body weight (kg) × base deficit (mmol/L) × 0.2, was given if the apH was less than 7.0 (in 10 HT and 11 NT). There was no relationship between any outcome measures and whether the piglet received THAM or not.

Mechanical ventilation was maintained as clinically required. HT and NT piglets were extubated after a median of 1.5 and 2.0 h, respectively. After 6 h, all HT and 13 NT piglets were extubated. Ampicillin and gentamicin were given twice daily, and no animals had signs of infection. Fluid (5% dextrose in 0.45% NaCl) was administered at 10 $mL \cdot kg^{-1} \cdot h^{-1}$ i.v. during preparation followed by 5 $mL \cdot kg^{-1} \cdot h^{-1}$ during the insult and for the first 24 h, after which artificial milk by bottle was introduced as tolerated. Serum glucose and electrolytes were checked and supplemented if needed. Target range for glucose was 2.6–10 mmol/L. MABP after the insult was maintained ≥40 mm Hg. Only one animal (HT) received dopamine,10 $\mu g \cdot kg^{-1} \cdot min^{-1}$, for 45 min during rewarming after HT. Bolus doses of 10% human albumin (median 10 mL/kg, range 7.5–20 mL) were given to 12 animals (seven NT and five HT) during preparation to treat hypotension after induction of anesthesia.

HT to a target T_{rectal} of 35°C was achieved within 20 min by removing external heating and placing rubber gloves filled with cold water (4–10°C) under and against the piglet's neck and body and was maintained by reducing incubator temperature to approximately 18°C and placing cold gloves as needed. After 24 h of HT, animals were rewarmed to 39°C over 6 h.

Blood gases were measured with a Radiometer ABL 4 blood gas analyzer adjusted for actual Hb value, and blood gas values

were corrected for body temperature. Glucose was analyzed with a standard hexokinase method, and cortisol was measured using the RIA procedure Coat-A-Count (Diagnostic Products Corp., Los Angeles, CA, U.S.A.).

Seizure detection. Electroconvulsive activity was defined as rhythmic high-voltage spike/wave activity at a frequency of less than 2 Hz lasting more than 20 s and with spike height more than twice the background voltage. It was detected by continuous observation of the two-channel EEG trace at 1 or 25 mm/s and later confirmed by examination of the paper trace or cassette recording by an investigator blinded to the temperature allocation of the pig. The onset of seizures was recorded and the proportion of time with seizures calculated for each 24-h period (Table 1). With recurrent seizures, the periods between seizures were included in the seizure time if they were shorter than the seizure periods.

The diagnosis of clinical seizures was made by observing clonic or tonic movements in a pig with simultaneous electrical seizure activity. If there was doubt about clonic/tonic movements, video film of the episode was reviewed by another investigator blinded to the temperature allocation. No anticonvulsant treatment was given.

Histopathology. At 72 h postinsult, the piglets were deeply halothane anesthetized and the brain perfusion fixed through the common carotid arteries with 4% phosphate-buffered paraformaldehyde. Three animals died prematurely due to cardiovascular consequences of the HI insult (one NT at 48 h and two HT at 33 and 46 h); their brains were also perfusion fixed. All three were seizing >50% of the time. Previously we have seen that animals that die prematurely after major insults and survive at least 24-h have developed maximal histologic damage (6, 8).

Typical lesions in this model are cortical necrosis in the bottom of the sulci (ulegyria), white matter lesions, cerebellar infarcts, selective Purkinje cell necrosis, and hippocampal lesions (8). Five areas of the brain were examined (hippocampus, cortex/white matter, cerebellum, basal ganglia, and thalamus) by a pathologist (E.M.L.) blinded to the treatment intervention and any clinical data. The severity of damage in 15 cross-sections per brain (frontal to rostral) was graded on a 9-step scale from 0.0 to 4.0 as previously described (8). All subjects had damage in at least one brain region.

Sham control animals. Three animals were fully instrumented and followed the same protocol as those randomized to 24-h HT followed by 48-h NT except that they did not undergo 45 min of hypoxia and were instead ventilated with room air.

Statistics. The SD for the mean pathology score without HT was known *a priori* as 0.95 (8) and was used to estimate a sample size of 15 to detect a difference of 1.0 in the pathology score with a power of 80% and a 2-sided p value of 0.05. We started with a total of 44 animals to allow for pilot experiments; $n = 2$, sham controls; $n = 3$ and experimental problems; $n = 3$ (see "Methods"). The piglets were randomized at the end of the insult by consecutively numbered sealed opaque envelopes in blocks of eight. Blocks were stratified according to whether the apH was above or below 7.0 at the end of hypoxia. Eighteen animals in each group were available for final analysis including the three that died prematurely. Mann-Whitney U test, t test, and simple regression were used as appropriate.

Table 1. *Subject and insult information and blood chemistry and hematology results throughout the experimental period. Values are mean (SD) or median with range as indicated*

	NT group $n = 18$	HT group $n = 18$
Postnatal age (h)	28 (11)	26 (13)
Weight (g)	1708 (180)	1522 (351)
Duration of LA EEG (min)	28.1 (7.2)	26.8 (8.6)
FiO_2 during insult (%)	5.9 (0.7)	5.8 (0.8)
EEG amplitude baseline (μV)	34 (4.6)	36 (5.2)
EEG amplitude 3 h postinsult (μV)	13 (3.5)	10 (3.3)
Number of animals with seizures	$n = 6$	$n = 7$
Onset of seizures after insult median (range) min	36 (9–527)	94 (27–613)
% duration of seizures (0–24 h)	51 (1–83)	26 (9–55)
% duration of seizures (24–48)	20 (0–72)	40 (10–98)
% duration seizures (24–72 h)	16 (0–35)	24 (7–100)

	Baseline −2 h		End of HI Time 0		24 h after HI End HT or NT		30 h after HI End rewarming		48 h after HI NT recovery		72 h after HI NT recovery	
	NT	HT	NT	HT	NT	HT	NT	HT	NT	HT	NT	HT
Hb (g/L)	7.8 (1.8)	8.6 (1.4)	—	—	7.4 (1.6)	8.3 (2.3)	7.0 (1.8)	7.3 (2.4)	7.1 (1.8)	6.9 (2.0)	7.1 (1.4)	6.7 (2.0)
Hematocrit	0.27 (0.05)	0.28 (0.05)	—	—	0.25 (0.06)	0.27 (0.07)	0.22 (0.05)	0.22 (0.08)	0.24 (0.06)	0.21 (0.07)	0.24 (0.06)	0.22 (0.07)
WBC (10^3/mm)	7.8 (4.3)	6.2 (3.7)	—	—	14.7 (6.7)	13.5 (6.9)	12.0 (5.9)	10.7 (7.7)	15.9 (9.9)	12.9 (6.7)	13.4 (5.8)	15.2 (11.5)
PLT (10^3/mm)	256 (74)	246 (50)	—	—	246 (119)	179 (84)	181 (58)	166 (92)	208 (95)	174 (153)	249 (115)	219 (159)
apH	7.44 (0.1)	7.45 (0.06)	6.98 (0.14)	6.99 (0.1)	7.55 (0.1)	7.55 (0.1)	7.49 (0.1)	7.49 (0.1)	7.46 (0.1)	7.48 (0.1)	7.44 (0.1)	7.45 (0.0)
PaO_2 (kPa)	16.1 (3.4)	17.8 (4.2)	7.8 (6.5)	5.3 (4.5)	14.4 (4.4)	13.5 (4.8)	15.9 (3.6)	16.8 (8.7)	15.1 (3.5)	13.6 (2.9)	14.9 (3.1)	14.1 (2.6)
$PaCO_2$ (kPa)	5.4 (1.0)	5.3 (1.0)	3.0 (1.2)	3.4 (0.8)	4.7 (1.1)	4.7 (1.7)	5.1 (0.8)	4.9 (1.1)	4.8 (1.0)	5.1 (1.0)	5.1 (0.8)	5.2 (0.9)
BE (mmol/L)	3.5 (3.8)	5.2 (7.3)	−24.5 (4.6)	−23.9 (4.1)	4.9 (4.3)	4.1 (3.7)	5.8 (4.2)	4.9 (5.3)	2.7 (5.6)	5.2 (3.4)	2.0 (5.9)	3.6 (5.5)
Gluc (mmol/L)	8.7 (2.1)	8.6 (1.8)	12.2 (4.5)	11.4 (4.4)	5.2 (3.6)	4.6 (4.5)	5.0 (1.9)	6.0 (4.9)	6.6 (7.1)	4.9 (2.8)	5.5 (2.8)	3.7 (1.5)
Na (mmol/L)	138 (5.7)	139 (5.4)	140 (8.4)	141 (4.8)	139 (7.1)	136 (6.1)	138 (6.3)	136 (7.4)	138 (7.0)	139 (9.6)	139 (9.9)	138 (6.9)

Results are given as mean ± SD or median with interquartile range or total range as appropriate.

RESULTS

Neuropathologic damage and temperature allocation. There were no differences in the neuropathology score or the distribution of the brain damage between the HT and NT groups (Fig. 1). Thalamus was significantly less damaged than the other regions of the brain ($p = 0.01$) as seen in this model (8). All animals were damaged in at least one region. In Figure 2, the relationship between individual pathology values in cortex/white matter and the duration of LA EEG during the 45-min insult is displayed ($r^2 = 0.54$, $p = 0.001$).

The duration of LA EEG (<7 μV) during the insult (mean HT 26.8 ± 8.6 *versus* NT 28.1 ± 7.2 min) was similar, as was the mean Fio_2 between the two groups (HT 5.8 ± 0.7 *versus* NT 5.9 ± 0.8) (Table 1).

Seizure activity and temperature allocation. Seven HT and six NT pigs developed posthypoxic seizures. The median total duration of time with seizures during the 3 d was similar in the HT (30%) and NT (29%) groups. The seizure activity tended to occur earlier in the NT group with median time until onset 36 *versus* 94 min ($p = 0.06$) (Table 1).

Neuropathologic damage and seizures. All regions of the brain were significantly more damaged in the group that developed seizures than in the group that did not. There was no significant difference between those that seized in the HT and NT groups.

In the piglets that seized, the median and total range pathology scores for hippocampus, cortex/white matter, cerebellum, basal ganglia, and thalamus were 4 (3.5–4.0), 4 (3.5–4.0), 3 (1.75–3.5), 3 (3–3.5), and 1.5 (1.0–2.5), respectively, in the HT group and 3.5 (3–3.5), 3.0 (3.0–3.5), 2.5 (1.5–3), 2.5 (2.5–3.0), and 1.0 (0–1.5) in the NT group. The HT animals that seized tended to be more damaged ($p = 0.06$). There was also a significant association between early onset of seizures and the severity of damage ($r^2 = 0.21$, $p = 0.003$). In the piglets that did not seize, the pathology scores (median and total range) for hippocampus, cortex/white matter, cerebellum, basal ganglia, and thalamus were 0.5 (0.0–3.5), 1.0 (0–3.0), 1.0 (0.0–3.0), 0.0 (0.0–2.5), and 0.0 (0.0–1.0), respectively, in the HT group and 2.25 (0.0–3.5), 1.5 (0.5–2.5), 1.5 (0.5–3.0), 0.0 (0.0–2.0), and 0.0 (0.0–1.0) in the NT group. These results were not significantly different between HT and NT.

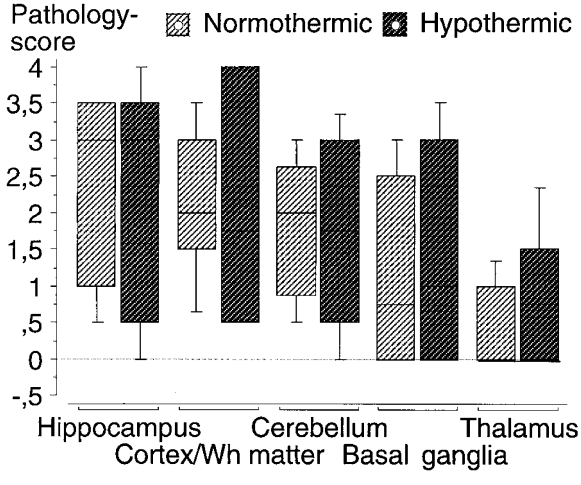

Figure 1. Shows the median pathology scores (and interquartile range) for the different areas of the brain in the HT group (24 h of 35°C T_{rectal} followed by 48 h of NT, 39°C) and NT group (T_{rectal} 39°C for 72 h).

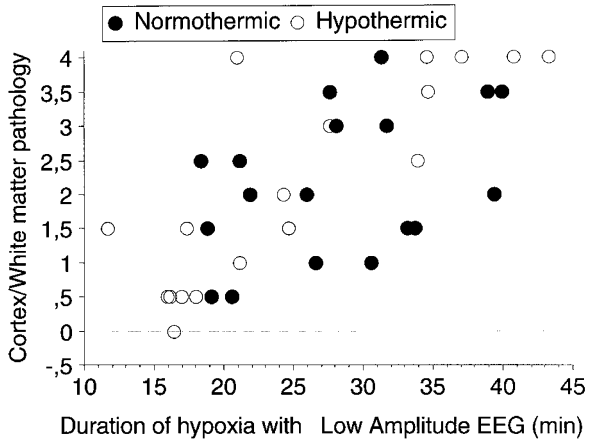

Figure 2. Shows the relationship between a 9-step pathology score (from 0.0 to 4.0) in cortex/white matter and the duration in minutes of the total hypoxic insult (45 min); the EEG amplitude was lower than 7 μV.

Two physiologic factors during the insult were the main predictors of later occurrence of seizures. The duration (median, range) of LA EEG during the 45-min insult was 34.5 (30.5–39) min in the group that developed seizures *versus* 21 (17.5–26.5) min in the group with no seizures ($p < 0.001$). The MABP during the insult was lower in the animals that went on to seize, in particular during the last 15 min of the insult, 38 (34.5–42.5) *versus* 50 (41–55) mm Hg in the group without seizures ($p = 0.005$). For the first 0–12 h after the insult, MABP, apH, or glucose was not related to the occurrence of seizures.

Hypothermic sham control animals. The brains were all normal with no damage in any region. The EEG at baseline, 3, and 72 h had normal background activity of 30–40 μV. The cardiovascular and biochemical parameters were also normal throughout with the following median values at baseline, after 24, 48, and 72 h, respectively: T_{rectal} °C, 39.0, 34.5, 39.0, 39.1; HR beats/min, 145 (anesthetized at baseline), 181, 190, 175; Hb g/100 mL, 6.7, 7.1, 7.5, 6.0; pH, 7.42, 7.40, 7.41, 7.42; P_{venous} CO_2 kPa, 50.1, 49.5, 51.3, 49.0; base excess (BE) mmol/L, 7.8, 6.2, 8.5, 1.1; glucose mmol/L, 8.0, 6.5, 6.0, 6.0.

Physiologic and biochemical data and temperature allocation. Table 1 shows that the two groups of pigs had similar physiologic and biochemical values before, at the end of the 45-min HI insult, and after 24, 48, or 72 h for Hb, hematocrit, white blood cell count, platelets, glucose, sodium, and arterial blood gas values. We analyzed specifically those factors during the insult thought to be associated with neurologic damage: temperature, HR, blood pressure, Fio_2, apH, $Paco_2$, Pao_2, and glucose. There was no difference between the NT and HT groups in any parameter we recorded during the insult (Table 1 and Fig. 3). After the insult, T_{rectal} was reduced by 4° in the

HT group for 24 h according to protocol. MABP or HR was similar in the HT and NT groups (Fig. 3).

Cortisol. In the HT group, plasma cortisol rose during the 24-h HT period to values 3 times the NT values (Fig. 4) and was significantly higher. After rewarming for 6 h, the HT values had normalized and were not different from the NT values. The decline in cortisol seen in the NT animals is normal for pigs from d 1 to 3 (20).

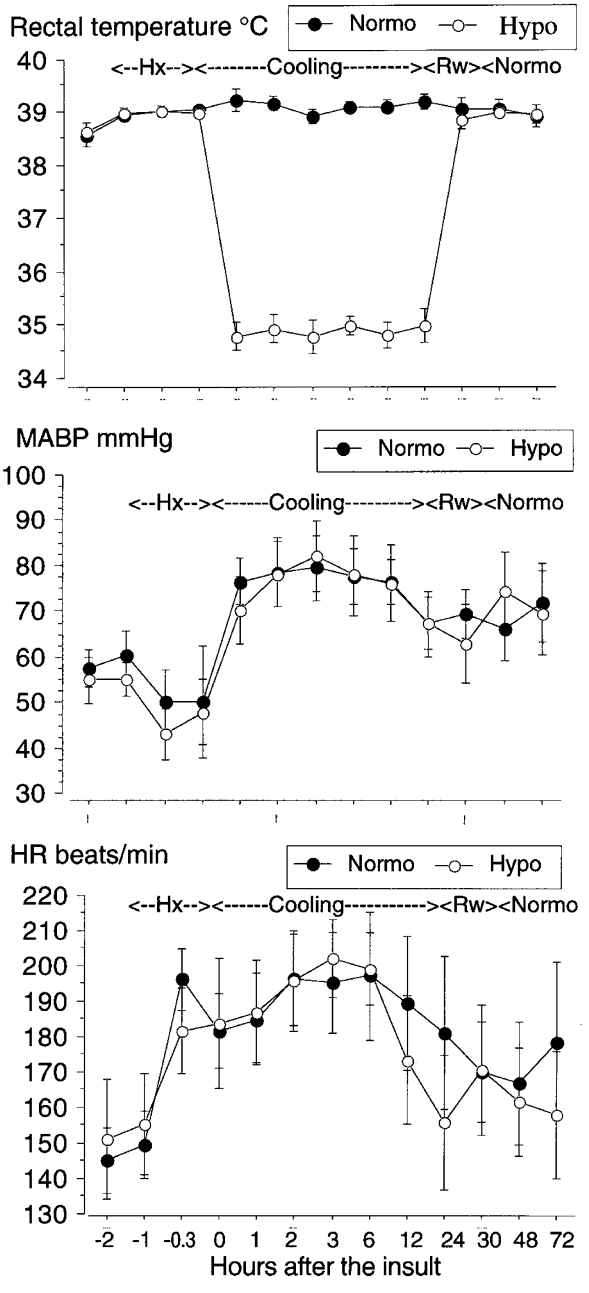

Figure 3. *Upper panel* shows the mean (±SD) T_{rectal} in the NT and HT groups throughout the whole experimental period. Only during induced HT is there a difference in temperature. *Middle panel* shows the MABP in the NT and HT groups. There is never a difference in blood pressure between the groups. The animals were anesthetized during preparation and baseline recordings (−2 h) until time 0 (end of insult), after which inhalation anesthesia was stopped. *Lower panel* shows there is no difference in HR between the two groups, although there is a trend for HR in the HT group to drop during the last 12 h of HT. *Rw* indicates rewarming.

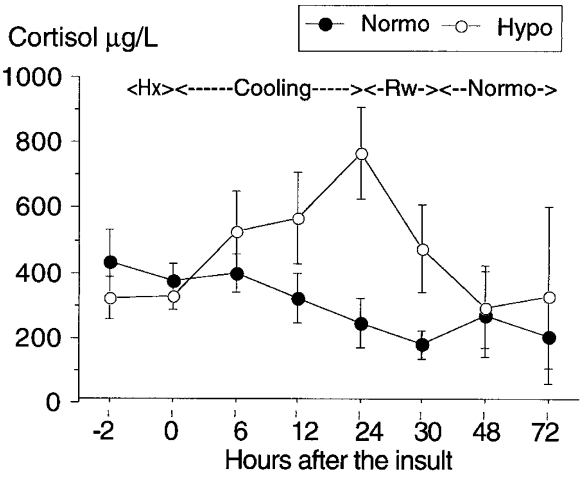

Figure 4. Plasma cortisol levels in the HT (n = 18) and NT (n = 18) groups of animals. Rw takes place from 24 to 30 h after the insult.

DISCUSSION

We found no neuroprotective effect of 24-h posthypoxic HT based on neuropathology or a reduction in the number of animals that developed seizures. This finding is in contrast with previous studies (21) that demonstrate neuroprotection in the newborn pig (1, 6), lamb (4), and rat (2, 3, 7) by use of posthypoxic HT lasting from 3 to 76 h. No study in newborn animals has compared the effect of different durations of HT. Moderate neuroprotection was found at 3-d survival after 3 h of 4° reduction (6). Twelve hours of 4° HT showed better protection in another piglet study (1). In both studies, the animals were fully anesthetized during HT. Based on these findings, we choose 24 h as a duration likely to be effective. The choice of a 4° temperature reduction was based on data from adult rats (22) and our pilot temperature study (16) as well as knowledge of possible adverse effects with moderate hypothermia (23).

Although one might suggest that the failure of neuroprotection could be connected to the absence of vessel occlusion in our model, we find that an unlikely explanation because our model does achieve a significant degree of ischemia secondary to hypoxic cardiodepression. We have found partial neuroprotection in our model with only 3 h of HT under anesthesia. Also, in a cerebral microdialysis study, we found a reduction in excitatory amino acids and citrulline/arginine ratio in the HT compared with the NT animals (18). HI brain injury in human newborns is not due to large vessel occlusion, and our model produces widespread brain injury with an anatomical distribution similar to that found in the full-term infant.

We speculate that the lack of protection in this study may be due to the stress of being cooled while awake, as previous studies on piglets that achieved protection were all performed on anesthetized animals.

The current study is, to our knowledge, the only experimental study in which the newborn pigs have not been anesthetized during posthypoxic HT.

We chose not to anesthetize the animals postinsult, as anesthesia is not routine practice with newborns after HI. Infants are usually sedated on clinical indication, *e.g.* if they are unsettled while being ventilated. The anesthetic could mask the

occurrence of seizures. We have not treated clinical seizures because treatment is relatively ineffective in humans (24) as well as in our model (6, 8). This group of drugs may be neuroprotective in themselves (25), and the administration may not be evenly distributed between the groups. Therefore, we followed the natural course of HI encephalopathy only supporting homeostasis.

Unsedated and conscious subjects that are cooled will try to maintain NT by increasing their metabolism by use of age- and species-specific strategies. Newborns with brown fat like the rat, rabbit, and man do not shiver but will turn on nonshivering thermogenesis (26, 27). Newborn species without brown fat like the pig will shiver (12, 13) and increase their metabolism by up to 300%. In awake adult humans, shivering increased whole body oxygen consumption by 110% when body temperature was reduced by 1.3°C (28), and shivering increased cerebral metabolism by 100% at HT temperatures in a small study on humans (29). The metabolic and temperature response to cooling if the subject is hypoxic is, however, different in newborns. In rabbits, mice, and rats, the temperature-preserving mechanisms are actively turned off and the animal becomes colder (26, 30, 31), which is seen to be a normal protective response by the hypoxic newborn. Burnard and Cross (32) measured T_{rectal} for 24 h after birth on vaginally delivered infants who were either normal at birth or moderately asphyxiated. Thirty minutes after delivery, the asphyxiated infants were 2°C colder than the healthy infants despite similar thermal care, and, after 20 h, their temperature was only 35.5°C.

In a fetal lamb HI model, cooling was provided by a coil of circulating cold water around the fetus. The fetus does not have any temperature regulatory responses itself and will not try to increase its heat production by shivering. We have, therefore, no measure of stress during intrauterine cooling in this model in which posthypoxic cooling for 72 h has shown excellent neuroprotection (4).

HT after unilateral carotid ligation and hypoxia in the unsedated 7- or 14-d-old rat has shown good neuroprotection (2, 3, 5). Newborn rats do not shiver but increase their metabolism by burning brown fat during HT. They do not increase their motor activity while cold after hypoxia but huddle together quietly and have a drop in HR corresponding to the HT (33). In adult brain trauma studies, the patients are always paralyzed and anesthetized during HT (9, 10, 34); hence, we have no comparative human data on HT without sedation.

Recent human pilot studies examined the efficacy and safety of total body cooling or selective head cooling during neonatal encephalopathy in infants who were not routinely sedated (35–38).

Stress, HT, and Neuroprotection

There is mounting evidence that stressful experiences exacerbate disease processes. Physiologic responses to stress include hypertension, tachycardia, and activation of the hypothalamic-pituitary-adrenal axis and release of cortisol. The brain, particularly the hippocampus, is a principal target organ for circulating glucocorticoids. The hippocampus is vulnerable to degenerative effects of stress (39). Swim stress, chronic handling stress, and limb restraint increase cortisol levels and hippocampal damage (40–42). Carlsson *et al*. (41) reported doubling of cerebral oxygen consumption in unanesthetized restrained rats.

In the same study, they blocked the adrenal response to stress by spinal anesthesia and eliminated the cerebral metabolic effects of restraint stress.

It is suggested that corticosteroids released during stress cause energy depletion in the brain, making it more vulnerable to exitotoxicity (43).

What evidence do we have that the cooled piglets were more stressed than the NT piglets? The main evidence is the steady increase in cortisol levels during HT, which fell immediately to normal levels on rewarming. Cortisol in piglets is a good marker of stress (44, 45). The lack of the expected reduction in HR during HT is an indirect marker of increased metabolic stress. In our previous studies of anesthetized piglets, the HR dropped by an average of 50 from 190 to 140 beats/min during mild HT (6, 16–18). In our clinical pilot study of posthypoxic cooling, nearly all the infants were sedated, and the HR was reduced from 145 to 100 beats/min during 72 h of cooling. The present study of unsedated piglets never showed a reduction in HR during HT.

In a study in which newborn piglets were kept in a cold (22°C) or warm (33°C) environment, the cold piglets were standing or moving 40% of the time compared with 8% in those who were kept warm. Those kept cold had higher plasma cortisol levels (46). In our study, the HT piglets were awake, more unsettled, and shivered more of the time than those that were NT.

Seizures

There are conflicting results as to whether HT reduces seizures *per se*. Anecdotally, maternal HT has been reported to stop eclamptic seizures and improve outcome in the infant (47). Also, HT has been reported to successfully treat status epilepticus (48). Reduced neuronal excitability was found when hippocampal slices were cooled (49, 50). In a trial of adults body-cooled to 33.5°C after brain trauma, the same numbers of patients were reported to seize in both the HT and NT groups (9). Also, in a trial of HT after out-of-hospital cardiac arrest, the same proportion seized in each group (51). In a fetal lamb cerebral HI model, 72 h of HT was not found to reduce the occurrence of seizures (4, 52). In our previous studies with short-lasting HT, the same proportion of animals seized in each group (6, 18, 19). In the current study in which no anticonvulsants were given and anesthesia was stopped immediately after the insult, there was a trend toward earlier onset of seizures in the NT than the HT animals. However, the HT group stopped seizing later; hence, the total time the two groups seized was the same (Table 1).

In conclusion, 24 h of mild HT without sedation failed to reduce neuropathologic damage or seizure activity in our newborn model of global hypoxia. This finding may be due to increased stress during HT. Multicenter trials of mild HT as therapy for newborn infants with neonatal encephalopathy have started, and routine sedation is not a part of the protocol.

If our findings apply to humans as well as pigs, we speculate that inadequate sedation of infants undergoing HT as therapy may block the protective effects of HT on the posthypoxic brain. We suggest that clinical and experimental research on the appropriateness of sedation during HT is urgent.

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