ORIGINAL ARTICLE

Changes in Amplitude-integrated Electroencephalograms in Piglets During Selective Mild Head Cooling After Hypoxia-ischemia

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Received Jun 3, 2013; received in revised form Sep 26, 2013; accepted Sep 30, 2013
Available online 17 January 2014

Key Words
aEEG; brain damage; hypoxia-ischemia; neonatology; neuroprotection; piglets

Background: Amplitude-integrated electroencephalogram (aEEG) is a simplified, alternative means of monitoring cerebral function and may be more useful clinically in some situations than conventional EEG. The aim of this study is to evaluate newborn piglets as an animal model to examine the effect of selective mild head cooling (HC) on aEEG after hypoxia-ischemia (HI).

Methods: Thirty-four piglets were randomly allocated to the following treatment groups: normothermic control group (NC, n = 7), selective HC control group (HC, n = 9), normothermic HI group (NHI, n = 9), and selective HC HI group (SHC-HI, n = 9). HI was induced by temporary occlusion of both carotid arteries and simultaneous reduction of the concentration of inspired oxygen to 6% for 30 minutes. Mild hypothermia (35°C) was induced after HI using a HC cap and was maintained for 24 hours. Changes in aEEG were monitored for 6 days after these treatments and the incidence of abnormalities analyzed. Physiological parameters were also measured during this period.

Results: In the two HI groups, animals exhibited severely abnormal aEEGs [continuous low voltage (CLV), burst-suppression, or flat tracing (FT)] 20 minutes after the beginning of HI.

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http://dx.doi.org/10.1016/j.pedneo.2013.09.012
1. Introduction

Perinatal hypoxic-ischemic brain damage (HIBD) is a major cause of perinatal mortality and long-term neurodisability. Evidence from both human and animal studies has shown that hypothermia provides neuroprotection from hypoxia-ischemia (HI) in adults and young/newborns, and it can reduce the incidence of cerebral palsy. Clearly, hypothermia is a promising treatment for perinatal HIBD.

Monitoring changes in electrocortical brain activity during HIBD can help assess the degree of brain damage and predict neurological outcome. For many years, conventional electroencephalography (EEG) has been used for intermittent and continuous assessment of brain function and for the prediction of neurological outcomes in infants. However, conventional EEG is of limited applicability in the neonatal intensive care unit. The use of multichannel EEG to monitor cerebral function in newborn infants who are in a critical condition is impractical. A simplified, alternative means of monitoring cerebral function is amplitude-integrated EEG (aEEG) and continuous assessment of brain function and for the prediction of neurological outcomes in term infants. However, conventional EEG is of limited applicability in the neonatal intensive care unit. The use of multichannel EEG to monitor cerebral function in newborn infants who are in a critical condition is impractical. A simplified, alternative means of monitoring cerebral function is amplitude-integrated EEG (aEEG).

Advantages of aEEG are that it is easy to use and analyze, shows less interference from artefacts such as muscle contraction, and positions the electrodes over the parietal zone, above an area known to be sensitive to ischemia. This recording approach has been shown to be one of the most accurate bedside methods for predicting neurological outcomes in term infants after HI. Indeed, this non-invasive technique has been increasingly used to identify infants suitable for hypothermic neuroprotection following severe HI.

To date, there is little information available regarding the use of aEEG to monitor changes in brain electrical activity in newborn animals after induction of HI and hypothermia. Therefore, we carried out an experiment in newborn piglets to examine aEEG and corresponding physiological data before and up to 6 days after the induction of HI and subsequent 24-hour mild hypothermia. We hypothesized that aEEG would be abnormal after the induction of HI and that mild hypothermia would promote normalization.

2. Materials and methods

2.1. Animals

Thirty-four healthy newborn white piglets of either sex were obtained from an experimental animal nursery in Shanghai. They were aged between 5 days and 7 days (term delivery) and weighed from 2.10 kg to 2.71 kg (mean: 2.35 ± 0.18 kg).

At 2 hours, the aEEG returned to normal in most of these animals. From 12 hours to 6 days, all animals in the NHI group exhibited severely abnormal aEEGs. Fewer animals in the SHC-HI group exhibited severe abnormal aEEGs during this time period, and four out of nine (44.4%) animals had continuous normal voltage (CNV) at 6 days.

**Conclusions:** Selective mild HC decreases the incidence of severe abnormal aEEGs at late times after HI in newborn piglets.
acidosis, but in the actual experiment, these adjustments were not needed for any animal. Animals were on mechanical ventilation with room air. No lung injury was observed; that is, animals had no difficulty breathing, and the results of the blood gas analyzer showed no CO$_2$ retention. On Day 7, they were extubated and sacrificed with an overdose of sodium pentobarbital.

2.3. Induction of acute cerebral HI

Cerebral HI was induced using a previously described technique. After surgical preparation and the stabilization period on the warmer bed, HI was induced by simultaneous temporary occlusion of the bilateral carotid arteries and abrupt reduction of the concentration of inspired oxygen to 6% for 30 minutes.

During HI, piglets received continuous physiological monitoring of MAP, heart rate (HR), arterial pH and base excess (BE), blood glucose, and electrolytes ($K^+$, $Na^+$, and $Ca^{2+}$). Physiological measurements were also taken during the subsequent 2 hours to 6-day time period. At the end of HI, the concentration of inspired oxygen was increased to 30%, and the occlusion of the bilateral carotid arteries was terminated so that reperfusion could occur.

2.4. Selective HC and temperature monitoring

Prior to HC, rectal temperature was maintained at 39°C in the two HC groups, as in the normal and HI groups. In animals subjected to HI and HC, HC began 2 hours after the hypoxic-ischemic period. In animals given HC alone, it was given immediately after the 2-hour postsurgical equilibration period. For HC in both groups, the head of the piglet was wrapped in a specially made thermostatically-controlled cooling cap with a network of circulating water channels (YJW608-04B Cooling Care System, Hengyang Radio Factory, Hengyang, Hunan Province, China). Circulating cooled water was automatically adjusted between 5°C and 24°C, as required after cooling started, until the nasopharyngeal temperature, a temperature that was shown to correlate well with cerebral temperature in a preliminary study, decreased to 35 ± 0.2°C. This temperature was maintained for 24 hours. Rectal temperature was continuously recorded using a temperature probe (Datex-Engstrom CS/3 monitor) (Hengyang Radio Factory) that was inserted 5 cm into the rectum. The rectal temperature was maintained at 36.0 ± 0.2°C using a servo-controlled radiant warmer bed. Twenty-four hours after the start of selective HC, the cap was removed and the animal was allowed to warm in room air without application of additional heating. For piglets in the two normothermic groups (NC and NHI), rectal temperature was maintained at 39°C throughout the entire study period.

2.5. aEEG recording and analysis

A cerebral function monitor (CFM 5022, Lectromed Devices Ltd, Letchworth Garden City, UK) was used for recording aEEG. Recordings were made at time 0 (during the 2 hour postsurgical equilibration period), 20 minutes (the middle of the HI period for those that had this treatment), at 2 hours (the period between HI and HC in those that had both treatments), and 12 hours, 24 hours, 48 hours, 72 hours, and 6 days after HI. Recordings at each time point were at least 2 hours in duration. Because the hypothermic head cap and the aEEG could not be used simultaneously, no recording was made at the 12 hour time point in the two selective hypothermia (HC and SHC-HI) groups. According to a previously described method, aEEG traces were assessed visually and classified into the following categories (Figure 1): (A) continuous background activity with voltage 10–25 μV (continuous normal voltage, CNV); (B) discontinuous background activity with voltage predominantly >10 μV (discontinuous normal voltage, DNV); (C) continuous background pattern of voltage below 10 μV (continuous low voltage, CLV); (D) discontinuous background pattern with periods of very low cortical activity (<5 μV), intermixed with bursts of higher amplitude (burst suppression, BS); and (E) mainly inactive (isoelectric tracing) of extremely low voltage (<5 μV; flat tracing, FT). All aEEGs were read by a single EEG specialist.

2.6. Measurements of physiological indices

Arterial blood samples were analyzed using a Nova analyzer (Nova Biomedical, Waltham, MA, USA). HR and MAP were monitored continuously using a critical care monitor (Datex-Engstrom CS/3).

2.7. Statistical analysis

Data are presented as mean and standard deviation and were compared between groups at each time point by one-way analysis of variance. Post hoc comparisons were made using Bonferroni corrections for pair-wise groups. The aEEG results are presented as count (%) and were compared between groups by Fisher’s exact test. All statistical assessments were two-sided and evaluated at the 0.05 level of significance. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

3. Results

No deaths occurred during the HI insult or during the following 6 days, and no convulsions occurred in any group. One animal died at the beginning of the modeling process. The baseline characteristics [sex, weight, HR, and MAP of the 4 groups were comparable and no significant difference in weight, sex, HR, or MAP was observed between the 4 groups (Table 1)].

3.1. Amplitude-integrated electroencephalograms

The aEEG results during the study period are summarized in Table 2. All animals had normal aEEGs at 0 hours. For the rest of the study period, as expected, all animals in the normal control group exhibited CNV, as did all animals in the hypothermic control group, except for five, which had DNV at 24 hours, but not prior to or afterward. In the two groups with HI (NHI and SHC-HI), all animals exhibited severe abnormal aEEGs at 20 minutes. In the NHI group, all nine animals were CNV prior to HI; 20 minutes after HI, four...
BS (44.4%), two CLV (22.2%), and three (33.3%) FT were observed, and no CNV or DNV; 2 hours after HI, six (66.7%) animals returned to CNV, and two DNV and one BS were observed. Similar results were observed during 12 hours—6 days after HI, with no CNV or DNV, but only BS, CLV, and FT observed. In the SHC-HI group, all nine animals were CNV prior to HI; 20 minutes after HI, six BS (66.7%) and three FT (33.3%) were observed, and no CNV, CLV, or DNV; 2 hours after HI, seven animals returned to CNV (77.8%), and two DNV (22.2%) were observed; 24 hours and 48 hours after HI, the majority of events were CLV (66.7%), followed by DNV (22.2% and 33.3%); 72 hours after HI, all animals had DNV; and finally 6 days after HI, four CNV (44.4%) and five DNV (55.6%) were observed (Table 2).

**Figure 1** Amplitude-integrated electroencephalogram waveforms in newborn piglets: (A) continuous normal voltage; (B) discontinuous normal voltage; (C) continuous low voltage; (D) burst suppression; and (E) flat tracing.
3.2. pH, BE, and glucose concentrations

The pH, BE, and glucose concentrations during the study period are summarized in Figure 2. At 20 minutes (that is, during the HI period) pH and BE were significantly lower and glucose concentrations significantly higher in the two HI groups compared with the two groups not subjected to this procedure (all comparisons \( p < 0.05 \)). pH levels were also significantly lower in the SHC-HI group compared with the NHI group (\( p < 0.05 \)). At 20 minutes, pH levels were 7.23, 7.08, 7.46, and 7.47 in the NHI, SHC-HI, NC, and HC groups, respectively. Corresponding BE concentrations were 14.14 mmol/L, 14.68 mmol/L, 2.20 mmol/L, and 2.19 mmol/L, respectively. Corresponding glucose concentrations were 18.71 mmol/L, 16.19 mmol/L, 7.16 mmol/L, and 7.38 mmol/L, respectively. From 2 hours onwards, there were no intergroup differences in pH or BE. From 2 hours to 36 hours, there were no intergroup differences in glucose concentrations; however, from 48 hours onwards, glucose concentrations were significantly lower in the HC, NHI, and SHC-HI groups compared with the NC group (all comparisons \( p < 0.05 \)).

3.3. HR and MAP

HR and MAP during the study period are summarized in Figure 3. There were no significant intergroup differences in HR at baseline 20 minutes or from 36 hours onwards. HR was significantly lower in the HC group compared with the NC and NHI groups at 2 hours, 12 hours, and 24 hours, and compared with the SHC-HI group at 12 hours and 24 hours (all comparisons \( p < 0.05 \)). HR was also significantly lower in the SHC-HI group compared with the NC and HC groups at 2 hours (both comparisons \( p < 0.05 \)). There were no significant between group differences in MAP throughout the study; MAP ranged from 67.0 mmHg to 72.4 mmHg.

3.4. Electrolyte concentrations

The electrolyte (Na\(^+\), K\(^+\), and Ca\(^{2+}\)) concentrations during the study period are summarized in Figure 4. All electrolyte concentrations remained stable in each of the four treatment groups throughout the study. There were no significant differences between the groups.

Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline comparisons between groups.</th>
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<tbody>
<tr>
<td></td>
<td>NC (n = 7)</td>
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<tr>
<td>Sex</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td>Male</td>
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<tr>
<td>Weight (kg)</td>
<td>2.31 (0.23)</td>
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<tr>
<td>Heart rate (beat/min)</td>
<td>200.43 (12.53)</td>
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<tr>
<td>MAP (mmHg)</td>
<td>67.14 (6.01)</td>
</tr>
</tbody>
</table>

Weight, heart rate, and MAP are presented as mean (standard deviation); sex is presented as \( n \) (%).

HC = head cooling; MAP = mean arterial pressure; NC = normothermic control; NHI = normothermic hypoxia-ischemia; SHC-HI = selective head cooling hypoxia-ischemia.

Table 2

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Effects of mild hypothermia on amplitude-integrated electroencephalograms after hypoxia-ischemia. *†</th>
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<tbody>
<tr>
<td></td>
<td>Time</td>
</tr>
<tr>
<td>NC (n = 7)</td>
<td>CNV</td>
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<tr>
<td>HC (n = 9)</td>
<td>CNV</td>
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<td>NHI (n = 9)</td>
<td>DNV</td>
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<td></td>
<td>CLV</td>
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<td>SHC-HI (n = 9)</td>
<td>BS</td>
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* Treatment groups: HC = selective head cooling without hypoxia-ischemia + nasopharyngeal temperature maintained at 35°C and rectal temperature maintained at 36°C; NC = normal temperature with rectal temperature maintained at approximately 39°C; NHI = normothermic temperature after hypoxic-ischemic insult + nasopharyngeal temperature maintained at 39°C; and SHC-HI = head cooling (mild hypothermia) after hypoxic-ischemic insult + nasopharyngeal temperature maintained at 35°C and rectal temperature maintained at 36°C.
† Amplitude-integrated electroencephalogram categories: BS = burst suppression; CLV = continuous low voltage; CNV = continuous normal voltage; DNV = discontinuous normal voltage; FT = flat tracing.
† Indicates a significant difference among the groups (\( p < 0.05 \)).
between group differences at any time point. Mean Na\textsuperscript{+}, K\textsuperscript{+}, and Ca\textsuperscript{2+} concentrations ranged from 136.22 mmol/L to 138.24 mmol/L, from 4.22 mmol/L to 4.62 mmol/L, and from 1.08 mmol/L to 1.14 mmol/L, respectively.

4. Discussion

In this study, we examined dynamic changes in aEEG after the induction of HI and during subsequent mild hypothermia in newborn piglets. We found that all animals exposed to HI exhibited severe abnormal aEEGs at 20 minutes, during the HI period. Thereafter, however, animals who also received selective HC for the induction of hypothermia (SHC-HI) exhibited fewer aEEG abnormalities after this treatment was given. Indeed, by 72 hours, no animals in this group exhibited severe abnormal aEEGs, whereas all animals who had HI without subsequent hypothermia (NHI) exhibited severe abnormal aEEGs from 12 hours onwards. These findings indicate that selective mild HC decreases the incidence of severe aEEG abnormalities.

We also studied physiological parameters during and up to 6 days after hypoxia/ischemia and HC. HR was decreased in the HC group for the first 2 days, and glucose was increased during hypoxia/ischemia in the two hypoxia/ischemia groups and very slightly in the last 2 days in the normal control group. No differences in MAP or plasma electrolytes were seen.

Figure 2  (A) pH; (B) base excess (BE); and (C) blood glucose concentrations before and during the study period. Treatment groups: HC = selective head cooling without hypoxia-ischemia + nasopharyngeal temperature maintained at 35°C and rectal temperature maintained at 36°C; NC = normal temperature with rectal temperature maintained at approximately 39°C; NHI = normal temperature after hypoxic-ischemic insult + rectal temperature maintained at 39°C; SHC-HI = head cooling (mild hypothermia) after hypoxic-ischemic insult + nasopharyngeal temperature maintained at 35°C and rectal temperature maintained at 36°C. * Indicates a significant difference compared with the NC group (p < 0.05). † Indicates a significant difference compared with the HC group (p < 0.05). ‡ Indicates a significant difference compared with the NHI group (p < 0.05).
In the current experiment, mild cooling (to 35°C) of the head for 24 hours was used. The degree of cooling that would produce optimum results depends on the species. Experiments with rats have used cooling to a rectal temperature of 32°C–35°C for 24 hours. Research with humans has used HC to 34°C–37°C for 72 hours. In a previous study, we found that HC to 32°C in piglets after HI produced no further improvement over cooling to 35°C. Thoresen also pointed out that within the clinical cooling range (33.5°C–37.0°C) temperature did not affect aEEG in animal experiments.

The severe aEEG abnormalities, CLV, BS, and FT, seen at 20 minutes in hypoxic-ischemic animals, are indicative of severe depression of cerebral function and signify acute hypoxic-ischemic damage to the brain. The second, later period of abnormal aEEGs is suggestive of progressive hypoxic-ischemic damage to the central nervous system and consequent impaired function. These two clear periods of abnormal aEEGs may reflect cerebral energy metabolism failure in the cerebral cortex, and they are consistent with findings from previous studies, suggesting that acute hypoxia can lead to initial and secondary failure of energy metabolism in the cerebral cortex. Indeed, there is evidence to suggest that brain cell energy metabolism recovers relatively quickly in newborn animals exposed to HI after resuscitation. Then, after a relatively stable period of 9–24 hours, oxidative phosphorylation becomes impaired, leading to secondary energy failure.

Previous research has shown that cerebral lactate production is increased during the 8-hour period after HI. Presumably, the increased metabolic requirements of the recovering brain cause a shift from oxidative to glycolytic metabolism. HC during this period significantly decreased cerebral lactate production, presumably because hypothermia reduced metabolic requirements. Our experiment covered a longer time period than 8 hours, however, and we did not collect data on lactate. The increase in glucose seen in our study during the HI period, in those that had this treatment, was probably due to a general stress reaction. The decrease in blood glucose at late times in NHI and SHC-HI rats was probably a result of the hypothermia, as it has been shown that hypothermia reduces blood glucose in patients with severe traumatic brain injury. It is speculated that the lower blood glucose at late times in the NHI group may be a long-term effect of the hypoxia/ischemia. In rats studied 2 weeks and 6 weeks after HI with or without subsequent hypothermia, a reduction in neuronal loss was seen with hypothermia treatment. From our results, it may be speculated that an improved matching of the brain’s metabolic needs with its oxygen supply and a lessening of neuronal loss caused by the hypothermic period contributed to the observed improvement in aEEG at 6 days in our experiment.

Findings from the present study in newborn piglets suggest that brain function deteriorates with time after the induction of HI; these are generally consistent with...

Figure 3 (A) Heart rate (HR); and (B) mean arterial pressure (MAP) before and during the study period. Treatment groups: HC = selective head cooling without hypoxia-ischemia + nasopharyngeal temperature maintained at 35°C and rectal temperature maintained at 36°C; NC = normal temperature with rectal temperature maintained at approximately 39°C; NHI = normal temperature after hypoxic-ischemic insult + rectal temperature maintained at 39°C; SHC-HI = head cooling (mild hypothermia) after hypoxic-ischemic insult + nasopharyngeal temperature maintained at 35°C and rectal temperature maintained at 36°C. * Indicates a significant difference compared with the NC group (p < 0.05). † Indicates a significant difference compared with the HC group (p < 0.05). ‡ Indicates a significant difference compared with the NHI group (p < 0.05).
the findings from our studies in human infants. Mild hypothermia has been shown to be one of the most promising treatments for HIBD. Findings from a previous study suggest that mild hypothermia protects brain tissue by alleviating further damage to mitochondria after ischemia reperfusion, improving energy metabolism, shortening secondary energy failure time, and preventing apoptosis.16 In this study, we found that HI led to marked suppression of aEEG waveforms and a predominance of BS and FT. This was particularly pronounced 24–48 hours after HI. These findings lead us to suggest that mild HC significantly increases the frequency of normal aEEGs, and in turn, promotes recovery from neuronal damage associated with HI.

Thoresen has shown that in infants with neonatal asphyxia, both aEEG recorded within 6 hours after birth and recovery time to normal aEEG, were good predictors of outcome at 18 months in both normothermic infants and infants treated with HC. Our study was not conducted for poor outcome prediction, so we cannot compare our results with those of Thoresen. In our animal study, we recorded aEEG over a longer time period than Thoresen and included physiological measurements. Instead of using outcome as an endpoint, we wanted to begin to explore whether aEEG measurements at later times might have clinical utility in monitoring recovery from neonatal asphyxia and for assessing the impact of different treatment procedures.

What is new in this research is recording the course of aEEG patterns in cooled versus non-cooled animals. What is also new, is the comparison of physiological data, including HR, MA, pH, BE, blood glucose, and electrolytes, both prior to and after hypoxia/hypothermia between NHI and SHC-HI groups. Our results may provide a feasible piglet model using aEEG to explore HI-related studies such as pharmaceutical applications.
Our study has a number of limitations. We only included a relatively small number of animals in each treatment group; hence, these studies should be repeated to confirm the observed findings. We did not perform histological examinations of brain tissue. We also did not assess the long-term consequences of the treatments because, due to budget constraints, we are unable to support and keep animals for a long time period. Clearly, further studies are warranted to assess the effects of mild hypothermia after HI on later brain and overall functioning.

In summary, the findings from our study of newborn piglets indicate that selective mild HC decreases the incidence of severe aEEG abnormalities after HI.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

References