·Original Article in English·

Effects of Moderate Hypothermia on Cerebral Energy Metabolism in Neonatal Rats with Hypoxic-Ischemic Brain Damage

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Abstract: Objective To study the effects of moderate hypothermia on cerebral glucose and ATP contents and mitochondrial succinate dehydrogenase (SDH) activity in neonatal rats with hypoxic-ischimic brain damage (HIBD). **Methods** Seven-day-old Sprague-Dawley rat pups were subjected to ligation of the left carotid artery followed by exposure to hypoxia environment of 8 % O_2 for 2 h, then they were randomly assigned into normothermic recovered group (IN group) and moderate-hypothermic recovered group (IH group). The sham-operated rats which were used as the control were assigned into the normothermic control group (NC group) and moderate-hypothermic control group (HC group). The contents of glucose and ATP and activity of mitochondrial SDH were assayed at 0, 2, 6, 24, 48, 72 h after HIBD. **Results** The cerebal glucose contents in the IN and IH groups were significantly lower than those of the NC and HC groups 0 h after HIBD. Two hours after HIBD, the glucose content recovered to normal level. The concentration of ATP and activity of SDH in the IN group decreased first, then they recovered gradually and reached a peak 72 hours after HIBD. After 6 hours or 2 hours of HIBD, the ATP content or SDH activity of the brain in the IH group were significantly higher than those in the IN group. The ATP concentration was closely related to the SDH activity (r = 0.515, P < 0.01). **Conclusions** Moderate hypothermia might protect brain tissue by inhibiting the decrease of SDH activity and increasing the synthesis of ATP. **[Chin J Contemp Pediatr, 2003, 5(3): 192-196]**

Key words: Moderate hypothermia; Cerebral hypoxia; Cerebral ischemia; Mitochondria; ATP; Glucose; Neonatal rat

亚低温对新生鼠缺氧缺血脑损伤能量代谢的影响

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[**摘 要**] **目的** 探讨亚低温治疗对缺氧缺血脑损伤(HIBD)新生大鼠的脑组织葡萄糖、ATP 及脑线粒体琥珀酸脱氢酶(SDH)活性的影响及意义。**方法** 将 HIBD 模型鼠随机分为缺氧缺血常温恢复组(IN)和亚低温干预组(IH),同时设常温对照组(NC)和亚低温对照组(HC)。各组动物在缺氧缺血结束后不同时间点(0,2,6,24,48,72 h)断头取脑,测定脑匀浆葡萄糖含量、ATP 含量及脑线粒体琥珀酸脱氢酶(SDH)活性。**结果** 脑糖在缺氧缺血 0 h明显低于对照组,缺氧缺血 2 h 后即恢复正常。IN 组脑 ATP 含量及 SDH 活性先行下降,以后逐渐恢复,72 h 达高峰;IH 组从缺氧缺血 6 h 后或 2 h 起 ATP 及 SDH 活性均显著高于同一时间点 IN 组。ATP 含量与 SDH 活性呈显著正相关(r = 0.515, P < 0.01)。**结论** 亚低温可减轻 HIBD 鼠线粒体 SDH 活性下降,改善能量代谢,增加脑 ATP 合成,从而保护脑组织。 [**中国当代儿科杂志,2003, 5(3): 192-196**]

[**关 键 词**] 亚低温;脑缺氧;脑缺血;琥珀酸脱氢酶;线粒体;ATP;葡萄糖;新生,大鼠

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The brain tissue's need for oxygen is very special in that the oxygen consuming for cerebral energy metabolism accounts for more than 1/2 of the total body oxygen consuming. Moreover, glucose is almost

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the only substance to supply energy for brain when it is oxygenated. But brain tissue has hardly storage of oxygen, glucose and ATP, so it has poor ability to endure hypoxia-ischemia and it is the most sensitive tissue to hypoxia-ischemia. In the newborns with hypoxic-ischemic brain damage (HIBD), because of the complexity of the mechanism, there is no sufficient effective treatment at present. Moderate hypothermia is one of the most promising therapy for HIBD[1~4], but the study for its mechanism is not sufficient. In the aspect of energy metabolism, we designed this experiment to study the effect of moderate hypothermia on cerebral glucose and ATP contents and mitochodrial succinate dehydrogenase (SDH) activity in the immature rats with HIBD.

Materials and methods

Animals and grouping

One hundred and twelve Sprague-Dawley rat pups were obtained from the Animal Center of Shanghai Experimental Center, Chinese Academy of Science.

The animal model was established according to Rice's[5] approach with some modifications. The rat pups were lightly anesthetized with ether inhaled, then the left common carotid artery (CCA) was ligated with 4.0 surgical silk and cut down between the ligations. After closure of the neck wound, the animals were allowed to recover from anesthesia. Then they were placed into airtight 500 ml glass container for 2 hours, in which the concentration of oxygen was 8 % and the concentration of nitrogen was 92 %. The temperature inside was about 33 ℃ by partially submerging them in water baths of 37 ℃. So the rats' rectal temperature (RT) was 37 ℃.

The rats of the control group were not placed into the hypoxic enviroment and their common cartioid arteries were only separated and not ligated.

Once the course of hypoxic-ischemia ended, they were assigned into two groups: the IN group, in which the rats recovered in water-bath box of 33 ℃, with 37 ℃ RT; IH group, in which the rats were placed in the environment of 29 ℃, with 32 ℃ RT. The rats of the control group were assigned into the NC group (RT = 37 ℃) and the HC group (RT = 32 ℃). All the four groups were fed with premature milk plus vitamins. 0, 2, 6, 24, 48, 72 hs after hypoxia-ischemia (HI) or separation of CCA, the rats were sacrificed by decapitation, with 5 - 6 rat pups in each time point.

Brain tissue preparation

The animals were decapitated on the ice. The left brain was dissected rapidly (less than 30 seconds) and put into the glass homogenizer with pre-ice-colded water 1.2 ml. Then it was homogenized manually for 15 times at 4 ℃. After homogenization, it was divided into three parts: 100 μl was used for protein quantification; 0.5 ml for the extraction of mitochondrial; the remaining was put into liquid nitrogen and subsequently deproteinised and the filtrate was stored at - 80 ℃ for measurement of brain glucose, ATP[6~9].

Preparation of mitochondria

Mitochondria was extracted from the homogenate of brain tissue at 4 ℃ by density-centrifugation and speed-centrifugation[9~11]. Protein in the fractions containing mitochondria was measured with the Lowry's method. The mitochondria was stored at - 20 ℃.

Measurement of biochemical indexes

The protein of the brain tissue homogenate was measured by Lowry's method. The brain glucose was measured by oxidase method[6,7]. The assay kit was obtained from Sigma company (ST. LOIS USA, G3660). The brain ATP was measured by specific enzymatic fluorometric techniques[7]. The activity of SDH of mitochondria was measured by spectrophotometer[12~14].

Statistical analysis

All the data were expressed as means ±S.D. ($\bar{x} \pm s$). ANOVA was used to analyze the comparison of muti-mean. Linear correlation was used to analyze the degree of relationship.

Results

General state

No scleredema or apnea occured in the course of hypothermia. No significant differences were found in sucking ability, sucking volume and body weight in animals of all groups. Because there was close correla-

tion between brain temperature (BT) and rectal temperature, the later is 0.5 ℃ lower than the former, we probed the RT instead of BT. The RT was maintained at about 32.5 ℃ in hypothermic group while it was at about 36.5 ℃ in normothermic group. The difference in temperature between normothermmic group and hypothermic group was 3 - 4 ℃.

Glucose concentration in the brain

Compared with the NC and HC groups, the brain glucose concentration in the IN and IH groups at 0 h after HI decreased significantly ($P < 0.05$). From the 2nd hour after HI, the brain glucose concentration of IN and IH groups recovered to normal level. See Figure 1.

Brain ATP content

Compared with the NC group, the brain ATP concentration in the IN group at 0, 2, 6, 24, 48, 72 h after HI accounted for 67 %, 64 %, 38 %, 45 %, 53 %, 145 % of those of the NC group. The brain ATP in the IH group at 0, 2, 6, 24, 48, 72 h after HI accounted for 67 %, 46 %, 66 %, 100 %, 127 %, 213 % of those of the HC group and the differences were obvious ($P < 0.01$ or 0.05). There was no difference between the IN and IH groups 2 hours after HI. From the 6th hour after HI, ATP concentration in the IH group was higher than that of the IN group ($P < 0.05$). See Figure 2.

SDH activity of mitochondrial in the brain

Compared with the NC group, the activity of brain mitochondrial SDH in the IN group 0 h after HI decreased significantly (accounted for 45 % of that of the NC group), then it decreased further. At the 6th hour after HI, the activity of SDH decreased to the lowest level (accounted for 24.7 % of that of the corresponding NC group), afterwards it increased gradually. 72 hours after HI, it reached a peak but was still lower than normal level (accounted for 49.9 % of that of the corresponding NC group). The activity of brain mitochondrial SDH in the IH group at 0, 2, 6, 24, 48, 72 h after HI accounted for 45 %, 37.2 %, 43.5 %, 51.3 %, 74.4 %, 97.4 % of those of the HC group, and the differences were obvious ($P < 0.01$). The activity of SDH in the IH group decreased to the lowest level at 2 h, then it increased slowly to the level of the HC group at 72 h after HI (accounted for 97.4 % of those of the corresponding HC group). From the 2nd hour after HI, the activity of brain mitochondrial SDH in the IH group was significantly higher than that of the IN group ($P < 0.05$). See Figure 3.

Comparison between two control groups

There were no significant difference in glucose, ATP concentrations and SDH activity between the two control groups (NC group and HC group) at all points of time ($P > 0.05$). See Figures 1, 2, 3.

The relationship between ATP concentration and mitochondrial SDH activity

The brain ATP concentration and the brain mitochondrial SDH activity were closely related: $r = 0.515$, $P < 0.01$ ($n = 112$).

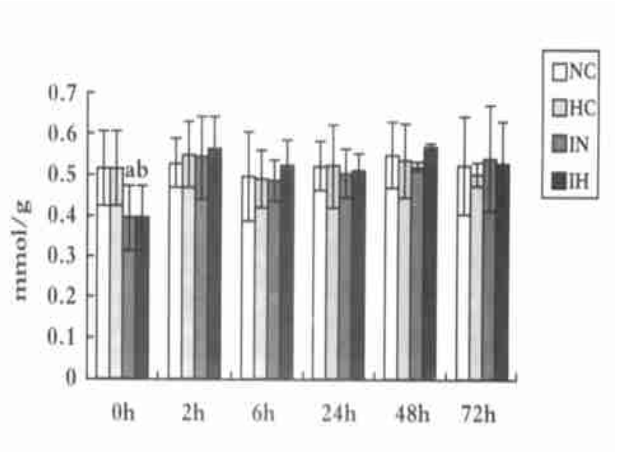

Figure 1 Brain glucose at different time points in the four groups

Note: a vs NC group $P < 0.05$; b vs HC group $P < 0.05$

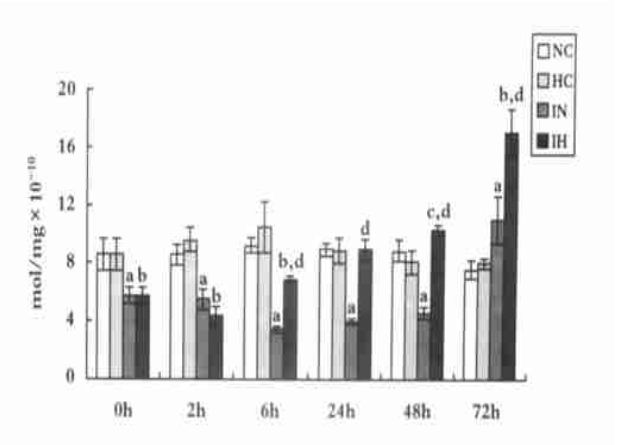

Figure 2 Brain ATP concentrations at different time points in the four groups

Note: a vs NC group $P < 0.01$; b vs HC group $P < 0.01$; c vs HC group $P < 0.05$; d vs IN group $P < 0.05$

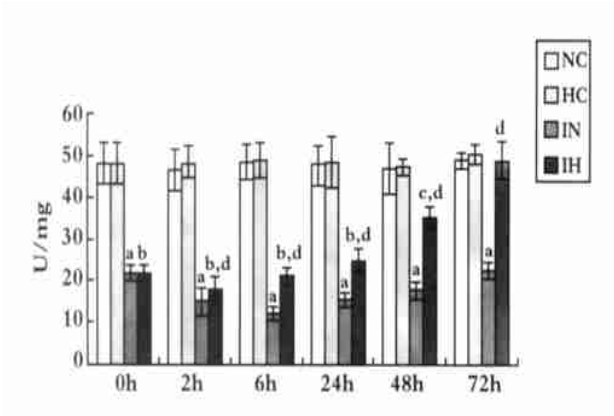

Figure 3 Activity of SDH at different time points in the four groups

Note: a vs NC group $P < 0.01$; b vs HC group $P < 0.01$; c vs HC group $P < 0.05$; d vs IN group $P < 0.05$

Discussion

The specificities of brain energy metabolism are in three aspects: ① the need for oxygen is very great; ②brain depends on blood glucose as the only supply of energy; ③ there is no energy storage of glycogen and ATP in the brain. And as we know, mitochondria is the most important place for energy supply by oxygenation. SDH is the speed - limited enzyme of tricarboxylic acid cycle (TAC) and also the mitochondrial characteristic enzyme, so the activity of SDH is in parallel with the mitochondrial function. In this experiment, glucose, ATP and the activity of mitochondrial SDH were used as the indexes of energy metabolism in the brain.

In this experiment, the two control groups (NC and HC groups) had no significant differences in general state, glucose level, ATP concentration and SDH activity, so the possible disadvantageous impacts of moderate hypothermia on rats were excluded. And we fed the four groups of newborn rat pups manually to avoid the difference caused by feeding.

The experimental data showed that, brain glucose, ATP and the activity of SDH decreased simultaneously after HIBD, but their recovery speeds were different. In the IN group, the recovery of brain glucose was the fastest, it has already recovered to normal level 2 hours after HIBD. But brain ATP concentration and the activity of brain mitochondrial SDH recovered comparatively slower. They decreased first, and then increased gradually. ATP rose gradually 24 hours after HIBD, it recovered to normal level 72 hours after HIBD. The activity of brain mitochondrial SDH rose slowly 24 hours after HIBD, but it was still lower than normal level 72 hours after HIBD. It suggested that in the course of ischemia/reperfusion, the function of mitochondria could recover after it was damaged. This was coincident with Sunshine's report[2]. The secondary neuronal damage of reperfusion occured 6 - 72 hours after the initial insult. Secondary cellular energy failure was related to the creation of oxygen free radicals in reperfusion. In the process of ischemia-reperfusion, the unoxygenated electron in the mitochondria connected with molecule O_2^- to become O_2, hydroxide free radical and other free radicals. These free radicals can inactivate enzymes and other proteins. So the activity of brain mitochondrial SDH kept on decreasing. However, in the IH group, brain glucose recovered to normal level 2 hours after HIBD, but the recovery of ATP concentration and the activity of SDH at the same point of time were faster than those of the IN group. Six hours after HIBD, ATP concentration and the activity of SDH began to rise. ATP concentration recovered normal 24 h after HIBD, and the activity of SDH recovered to normal 72 h after HIBD. These indicated that moderate hypothermia protected the brain tissue[2] by alleviating the further damage of mitochondria after ischemia-reperfusion, improving energy metebolism and preventing apoptosis[2].

This study showed that brain ATP recovered gradually after HIBD. It was correlated to the mitochondrial SDH activity, but not at the same speed. The recovery of ATP was faster than that of SDH activity. 72 hours after HIBD in the IN group, the activity of SDH didn't recover to normal, while the ATP level increased to normal. This was coincident with Wiegand's[15] report. The reason might be in that there are many factors impacted on the synthesis of ATP such as the activity of the enzyme of mitochondrial respiratory chain and ATP synthase and the ATP consumption rates et al.

In conclusion, this experiment showed that moderate hypothermia can protect brain by inhibiting the decrease of the activity of mitochondrial SDH and

increasing the ATP synthesis.

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·消息·

新生儿脑损伤学习班暨研讨会通知

(国家级继续医学教育项目 项目编号:2003 - 06 - 03 - 001)

为了提高新生儿科和产科医师对新生儿脑损伤的理论和临床认识,提高早期诊断的能力和治疗水平,经卫生部继续医学教育委员会批准,由湘雅医院儿科主办"新生儿脑损伤学习班"。该班由全国新生儿专家授课,同时由中国当代儿科杂志社举办新生儿脑损伤研讨会。内容涉及 HIE,颅内出血,胆红素脑病,遗传性代谢病所致的脑损伤,换血治疗,高压氧治疗,亚低温疗法,脑内移植治疗的实验研究和新生儿脑损伤的监测等等。欢迎新生儿科和产科医务人员参加。有学术论文者中国当代儿科杂志社将择优刊登,参加学习者经考核合格的可取得国家级 I 类学分 16 分。如有兴趣参加者可向以下地址索取正式通知。会议时间定为 2003 年 10 月上旬。

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