EFFECTS OF SELECTIVE HEAD COOLING ON BRAIN CELL MEMBRANE ACTIVITY DURING POSTISCHEMIC REPERFUSION

Duan Manlin 段满林, Li Dexin 李德馨 and Xu Jianguo 徐建国

Objective To examine the effect of selective head cooling (SHC) on brain cell membrane activity involving ATPase, phospholipase A_2, content of total membrane phospholipids during postischemic reperfusion, so as to elucidate the possible underlying mechanism on resuscitating effect of SHC.

Materials and Methods Complete cerebral ischemia (CCI) was induced by the four-vessel model. 56 New Zealand rabbits were allocated randomly into two groups: non-ischemic control group had 30, 180 and 360 minutes reperfusion after CCI (n = 8); and SHC group with the same ischemic-reperfusion insult were all treated with SHC (28℃, surface cooling method). Changes of Na^+, K^+-ATPase, Ca^{2+}, Mg^{2+}-ATPase, phospholipase A_2, total phospholipids of brain cell membrane were observed. Comparison of data between two groups was made by Students' t test.

Results Compared with non-ischemic controls following 30 minutes CCI, activities of Na^+, K^+-ATPase stepwisely decreased at 30, 180 and 360 minutes, Ca^{2+}, Mg^{2+}-ATPase dropped at 180 and 360 minutes, phospholipidase A_2 increased markedly at 30, 180, 360 minutes, and total phospholipids decreased at 180 and 360 minutes reperfusion ($P < 0.01$). Selective head cooling inhibited all the above changes significantly ($PP < 0.01$).

Conclusion The results suggest that selective head cooling initiated soon after reperfusion is beneficial for brain cell membrane function recruitment, which provides favourable effects on the damaged but still remediable brain cells for their resuscitation.

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It is well known from both clinical experiences and animal experiments that hypothermia instituted prior to and during circulatory arrest can ameliorate cerebral reperfusion damage and improve neurological outcome. Beneficial effects of selective head cooling (SHC) have been consistently demonstrated by us in cardiopulmonary cerebral resuscitation since 1962. More than 30 patients have been successfully resuscitated with this method in our hospital,[1,2] but the mechanism is still controversial. Brain cell membrane (BCM) dysfunction which has been implicated in the pathophysiology of brain ischemic damage and plays a critical role in the sequences of events leading to brain cell death following ischemic-reperfusion damages.[3] The aim of this study was to examine the influences of SHC on some BCM functions.

MATERIALS AND METHODS

New Zealand rabbits (n = 56) of both sexes weighing 1.8-2.2 kg, aged 3-4 months, were fasted for 12 hours before experiment. The animals were anesthetized with i.v. amobarbitol (6mg/kg) and fentanyl (1 μg/kg). Tracheotomy was performed after pancuronium (0.1 mg/kg), and mechanical ventilation was instituted. Tidal volume and rate were adjusted to keep PaO_2 above 13.3 kPa and $PaCO_2$ 4-6.7 kPa.

Catheters were advanced from a femoral artery and vein for mean arterial pressure (MAP) monitoring, blood sampling and injection of drugs. A small hole was drilled on the cranium for placing the temperature probe, the needle probe was inserted into the occipital lobe. Ligatures were gently placed around four vessels,

Department of Anesthesiology, Jinling Hospital, Nanjing 210002 (Duan ML, Li DX and Xu JG)

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i.e. the left subclavicular artery, the branchiocephalic trunk, and bilateral internal thoracic arteries.

Induction of cerebral ischemia. After about 30-minute stabilization of physiologic variables, the preischemic MAP was adjusted to 12 kPa by phlebotomy. Complete cerebral ischemia (CCI) was performed by tightening the ligatures around the four arteries for 30 minutes, and MAP was simultaneously decreased to 6.7- 9.3 kPa by blood letting or rejecting. By removing the ligatures, cerebral reperfusion was performed, and MAP was maintained at 10- 15 kPa throughout the entire reperfusion procedure by infusion of blood, Ringer solution without glucose, norepinephrine (2 μg/ml, as needed).[4]

Temperature monitoring and head cooling. The thermocouples were rapidly responding, and calibration of each probe was performed daily before study. The animal's normal brain temperature (Tb) was 38℃, and rectal temperature (Tr) 37℃. During reperfusion periods, selective head cooling was achieved by immersing the animal's head into ice water, and Tb was decreased to 28℃ for about 30 minutes. Tr was kept intuitively at the level not lower than 34℃ by adjustment of the heating lamps under experimental table.

Animal groups. The rabbits were allocated randomly into two groups (Table 1). Control group was kept normothermic throughout, subgroup 0 served as non- ischemic control; in subgroups 1, 2, 3, reperfusion lasted 30, 180 and 360 minutes respectively before being sacrificed. Animals in the SHC group, which suffered the same ischemic- reperfusion insult, were all treated with selective head cooling.

Table 1. Animal groups for comparison

Items	Control				SHC		
	0	1	2	3	1	2	3
Ischemic time (min)	0	30	30	30	30	30	30
Reperfusion time (min)	0	30	180	360	30	180	360
Number of animals (n)	8	8	8	8	8	8	8

BIOCHEMICAL STUDY

About 50 mg cortical tissues of the temporal lobe were taken at 0℃ and then preserved at - 150℃ liquid nitrogen. Brain cell membrane was obtained and the activities of ATPase on BCM were measured by Palmer's method,[5] and one unit of ATPase was expressed as 1 μmol Pi · mg (prot)$^{-1}$ · min. The activities of phospholipidas A_2 (PLA_2) were measured titrimetrically by Ziev's method,[6] 0.01- 0.001 mol HCl were titrimetrical solution, one unit of (PLA_2) was expressed as 1 μmol HCl · ml^{-1} · min.

Statistical analysis. Values of recorded variabls were expressed as $\bar{x} \pm s$. Comparisons between data of the two groups were made by analysis of Student's *t* test. *P* values were considered significant if they were less than 0.05.

RESULTS

ATPase in control group. Compared with non- ischemics, the activities of Na^+, K^+ - ATPase stepwisely decreased at 30, 180 and 360 minutes, Ca^{2+}, Mg^{2+} - ATPase decreased markedly at 180 and 360 minutes of reperfusion following 30 minutes CCI ($P < 0.01$); the reductions were not seen in the SHC group. The differences were statistically significant ($P < 0.01$, Tables 2, 3).

Table 2. The activities of Na^+, K^+ - ATPase (U) on BCM in two groups

	Control	SHC
0	13.24±0.64	
1	9.71±0.67*	12.36±0.91△
2	8.12±0.53*	13.76±1.13△
3	7.64±0.49*	13.13±0.69△

* Compared with subgroup 0; △compared with control group; $P < 0.01$.

Table 3. The activities of Ca^{2+}, Mg^{2+} - ATPase (U) on BCM in two groups

	Control	SHC
0	11.67±0.61	
1	10.43±0.87	10.62±0.57
2	8.04±0.59*	10.64±0.72△
3	7.27±0.55*	10.56±0.50△

* Compared with subgroup 0; △ compared with control group; $P < 0.01$.

PLA_2 in control group. The activities of PLA_2 increased significantly at 30, 180 and 360 minutes of reperfusion ($P < 0.01$), as compared with nonischemic controls. This increase was not seen in the SHC group ($P < 0.01$) as compared with the control group (Table 4).

Table 4. The activities of phospholipidase A_2 (U) on BCM in two groups

	Control	SHC
0	14.83±0.85	
1	20.73±1.82*	14.65±1.46△
2	62.97±4.35*	20.99±3.30△
3	23.34±1.73*	20.20±1.21△

* Compared with subgroup 0; △ compared with control group; $P < 0.01$.

Total phospholipids Compared with non-ischemic control, total phospholipids were decreased at 180 and 360 minutes of reperfusion ($P < 0.01$) in the control group. The reductions were not seen in the SHC group as compared with the control group ($P < 0.01$, Table 5).

Table 5. The contents of phospholipids on BCM in two groups [μmol/mg(prot)]

	Control	SHC
0	0.2110±0.0052	
1	0.2028±0.0051	0.2090±0.0114
2	0.1553±0.0148*	0.2150±0.0165△
3	0.1800±0.0029*	0.2089±0.0069△

* Compared with subgroup 0; △ compared with control group; * * △△$P < 0.01$

DISCUSSION

ATPase in the brain cell membrane is essential to the intracellular homestasis, metabolism, neurotransmittors release, etc. They can transport actively Na^+, K^+, Ca^{2+}, and Mg^{2+} ions. Active transport of Na^+ and K^+ ions accounts for about half of the energy consumption of the cells. Therefore, they are vital to the brain cells.[3]

A few munites after CCI, ATP is depleted completely and transport of Na^+, K^+, Ca^{2+} fails, then failure of brain cell membrane function develops. This is one of the passways to cause cerebral reperfusion damage.[3] Mg^{2+} transport system is associated with Ca^{2+} transport system. Intracellular Ca^{2+} accumulation and Mg^{2+} leakage were demonstrated after reperfusion in our experiments.[7] It is possible that the Mg^{2+} transport failure may also play a role in the development of brain cell membrane function failure.

Cerebral blood reperfusion does not necessarily promote the recovery of brain cell membrane functions, but may induce reperfusion damage such as inactivation of ATPase, lipids perioxidations, etc.[8] This study showed that ATPase is inactivated after reperfusion. The inactivation may be related to the following factors:[5,8] 1) brain cellular energy-debt state after reperfusion; 2) accumulation of radicals and lipids peroxides; and 3) intracellular acidosis.

It was demonstrated that SHC not only ameliorated the activities of ATPases, but also made them return to normal. These results suggest that head&cooling promotes the recruitment of brain cell membrane functions by membrane stabilization. The mechanisms responsible for the beneficial effects may include minimized energy failure, reduced ATP consumption, inhibited lipids perioxidations and depressed intracellular acidosis.

Activation of membrane-bound PLA_2 has several effects Xon brain cells, which result in destruction of brain cell membrane. Such degradation of brain cell membrane inactivates membrane-bound enzymes (such as ATPase). The products formed by PLA_2, free fatty acids and lysophospholipids can disrupt brain cell membrane and are cytotoxic.[9] It was demonstrated[10] that PLA_2 is activated during reperfusion, accompanied by degradation of membrane phospholipids and that free fatty acids and PLA_2 play a role in the reperfusion injury of brain cell and the changes of free fatty acids and PLA_2 are related to the accumulation of intracellular Ca^{2+}. These results support the following hypothesis: brain cell membrane dysfunction may be triggered and advanced during reperfusion, then structural damage develops with repeated attack of other cytotoxics, resulting in irreversible brain cell death.

The results showed that SHC inhibited the activation of PLA_2 and degradation of total membrane phospholipids, possibly because of the reactivation of ATPase, and decrease in intracellular calcium accumulation (because membrane-bound PLA_2 has an absolute requirement for calcium). Attenuation of degradation of phospholipids may also relate to the inhibition of lipids perioxidations, and acceleration of free fatty acids re-esterifying into lipids after minimization of energy failure by head cooling.[10]

The results show that SHC initiated soon after reperfusion promoted the recruitement of membrane

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functions by accelerating the recovery of ATPase activities and preventing the structural disruption of brain cell membrane through significant inhibition of the activation of PLA_2 and the degradation of membrane phospholipids. We found that SHC minimized the hypermetabolic state during the early stage of reperfusion[11] and ameliorated the development of brain edema, the disorders of excitatory amino acids and neuropeptides release (unpublished data). It is concluded that SHC initiated soon after reperfusion has beneficial effects on brain cell membrane function recruitment, and provides some favourable conditions for damaged but remediable brain cells to recover. SHC is more effective than certain drugs such as thiopentone in BRCT- I and calcium antagonists in BRCT- II.

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Comparison of the Three Methods for Estimating the Dry- Weight of Hemodialysis Patients Jiang Fen, Bi Zengqi, Bao Yuhong, et al. Department of Nephrology, Peking Union Medical College, PUMC Hospital 100730. Chin J Int Med 1996; 35(2).

In order to find the best method for estimating the dry- weight of hemodialysis (HD) patients, we compared the three methods used, i.e. bioelectrical resistivity (ρ), plasma cGMP (cGMP) and bromide (Br) methods. The results showed that the extracellular fluid volume per unit body mass (EFV/mass) determined with ρ was negatively correlated with that determined with Br (r = - 0.7602 for normal controls and - 0.5293 for HD patients, P < 0.05). However, plasma cGMP concentration was neither correlated with EFV/mass (r = 0.3724 for normal control and 0.2538 for HD patients, P > 0.05) nor with ρ (r = 0.5210 for normal controls and 0.2106 for HD paitents, P > 0.05).

These results suggest that the bioelectrical resistivity dry- weight method is more accurate than cGMP method and moreover it is easier to perform than the Br method.