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MILD BRAIN HYPOTHERMIA: POTENTIAL FOR NEURONAL PROTECTION AND RESUSCITATION AGAINST ISCHEMIC DAMAGE

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SUMMARY

• *Although hypothermia as a means of neuronal protection and resuscitation after ischemic damage has a history of approximately four decades, extensive studies on the mechanisms, effects and methods of mild hypothermia at no less than 32°C have been started only in the last decade, both in basic and clinical fields. In experiments on rodents, postischemically introduced hypothermia, even as late as several hours after reperfusion, which was main tained for one day followed by a slow rewarming, definitely rescued hippocampal neurons against damage. Hypother mia appears to have a much greater potential for cerebral resuscitation than any chemical proposed so far for this purpose. The mode of action of hypothermia is apparently nonspecific and multifocal in widely progressing cascade reactions in ischemic cells, including (i) suppressing glutamate surge followed by (ii) intraneuronal calcium mobilization, (Hi) postischemic sustained activation of glutamate receptors, (iv) dysfunction of blood-brain bar rier, (v) proliferation of microglial cells, and (vi) produc tion of superoxide unions and nitric oxide in microglial cells, and of activator protein-1 in ischemically vulnerable regions like hippocampal CA1. Recent clinical trials of mild hypothermia have revealed significantly beneficial out-comes along with an accumulation of knowhows on various techniques and treatments. Large scale randomized studies involving multiple institutions as well as exchanging ideas are needed for further development of hypothermia treatment.* ***(Biomed Rev 1997; 8: 23-36)***

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INTRODUCTION

• More than half a century has passed since human refrigeration was first introduced (1). Soon afterwards, the potential use of hypothermia for neuronal protection and resuscitation has been suggested from both experimental and clinical researches (2, 3). However, a variety of confounding side effects, such as cardiovascular dysfunction, and severe infections, have hindered hypothermia from being firmly established as a treatment for brain injury or stroke (4-9). Moreover, a lack of animal studies has resulted in little basic information on when, to what degree, or how long body tem perature can be safely lowered and maintained. This might be another reason for hesitations on the hypothermic treatment. It was only a decade ago when a milestone report for hypo thermia appeared on experiments with rodents in which slight lowering of body temperature to 33°C produced a marked protection of central neurons against ischemic damage (10). This report would be a signal fire for the second wave of human hypothermia for protection or resuscitation of ischemic brain. Since then, basic experiments have been widely carried out on the rat (11-28), and the gerbil (29-35). In these models, it is confirmed that mild hypothermia, even when introduced as late as several hours postischemia, is significantly more effective than any chemical proposed so far for protection of

neurons against ischemic degeneration (36-45). At the same time, clinical applications of mild hypothermia for patients in acute stages of brain injury or stroke have been performed, mainly in the USA and Japan (46-55). Although the mentioned side effects and limitations of application are still problems to be solved, indications for anesthesia, applicable clinical conditions, methods for temperature lowering, maintenance and rewarming, monitoring, and countermeasures for side effects have been steadily developed (56).

In this review, we briefly summarize the development and technique of hypothermia treatment for ischemic brain injury, and discuss the problems to be solved on both basic and clinical fields.

HISTORICAL BACKGROUND OF HUMAN REFRIGERATION

• Local cooling of the body surface have been widely employed as a folk remedy since the earliest days of medi cine. Hippocrates in Cos described the usefulness of local cooling with snow and ice before operation for its analgesic nature (57). However, it was little more than half a century ago when whole body cooling was first introduced, thus opening the new era of human refrigeration as a clinical countermeasure (1, 58). These authors put forth the idea that when body temperature is lowered, proliferation of malignant tumors and hence development of tumorigenic pain might be suppressed. In some cases, they designed a brain probe which was con nected to a refrigerator. Since their first patient in 1938, 169 cases in total were treated by hypothermia, with a mortality at 11.2%. Considering that all patients were in the terminal stage of their cancers, and that medical treatments were less mod ernized and antibiotics were not available, this pioneering work appears to have had a satisfiable and encouraging out come. The degree was generally 33°C, but some cases were as low as 22°C. Fay (59) summarized from these experiments that the reversible limit of hypothermia was 24°C, and that hypothermia at 29-32°C could be maintained for 10 days, a surprising result from the present standpoint. Soon after wards, Talbott (60) applied prolonged total body hypothermia to schizophrenia patients, and obtained striking and sustained improvements in 4 out of 10 trials. Further, McQuiston (61) proposed a total body cooling during operations on children with heart disease, and Bigelow *et al* (62) confirmed that hypothermia at 20°C reduced oxygen demand, cardiac output and metabolic rate to 15% of their values at normal tempera ture. Based on these findings, the possibility of open chest surgery under hypothermic conditions was strongly suggested (63). To explore this possibility, Parkins *et al* (64) installed a cooling bypass at the common carotid artery in dogs, in order to selectively cool down the cerebral circulation, and then examined the critical temperature at which central dysfunc tion began to occur; the dogs could tolerate hypothermia up to 12°C, and subsequently recovered without any failure of higher functions. Rosomoff and Holaday (65) analyzed the cerebral blood flow and oxygen consumption of dogs and found that both were reduced by 6.7% for every 1°C decrease in the temperature range of 25-35°C. It was shown that occlusion of the middle cerebral artery produced smaller cerebral infarctions at lowered temperature, which appears the first report on the brain resuscitating capability of hypothermia (66). Hence, a question was raised as what was the minimum temperature that mammals could tolerate (67); Gollan *et al* showed that dogs could survive cooling to 1.5°C (68). In 1955, a symposium entitled "The Physiology of Induced Hypothermia" was held in the USA, where wide range of basic findings were presented and discussed (69-71). Clinical studies were also described, wherein a significant reduction in paraplegia was obtained after surgical dissection of thoracic aorta aneurysms under hypothermia, while high indices of spinal cord damage were observed under normothermic operation in similar cases (72). Hirsch and Muller (73) made a rabbit model for transient global ischemia, and found that, histologically, the full resuscitation of cerebral neurons was obtained even after 40 min of ischemia at 25°C, while only 8-10 min was acceptable at the normal temperature. Two reports strongly suggested to us the ability of hypothermia to protect or resuscitate the brain in humans; both were 5 years old boys in Norway, who drowned in a frozen river or lake, one for 22 min (74), the other for 40 min (75), before being rescued and transported to the hospital. Both patients had no vital signs for more than 2-3 hours, but showed gradual recovery in intensive care over the next few days, and finally left the hospital without any central disturbances; rectal temperature in the second case was 24°C at the time of the rescue (75). At this temperature, cerebral blood flow, heat production, and ATP expenditure should be much less than those at normal temperature, and there should be virtually no firing of neurons, creating a state very similar to deep anesthesia. Other reports also described beneficial cerebral effects of hypothermia, which attracted considerable attention to the possibility of introducing hypothermia into clinical use as a treatment for cerebral injuries (76-80). There has been a growing body of evidence which indicates that hypothermia protects the brain against the harmful effects of a reduction in oxygen supply (81-84), barbiturate showing a similar protection (85, 86). The suggested mechanism was that hypothermia shifts the oxyhemoglobin dissociation curve to the left, preventing a rightward shift as result of acidosis, which could maintain a high arterial TO_2 at a given PaO_2, and a reduced $CMRO_2$ along with decreased , cellular energy requirement (84).

• Small animal models of ischemia

As described in the previous section, a report by Busto *et al* (10) has opened a second era for hypothermia studies. They j

observed in a four-vessel-occlusion model of the rat that lowering of body temperature by 2-4°C resulted in a significant resuscitation of hippocampal CA1 neurons, known to be extremely vulnerable to ischemia, in contrast to the delayed neuronal death that occurred at 37°C (87). In spite of the cerebral protection provided by hypothermia, hyperthermia was clearly shown to increase damage in a transient ischemic model of the rat (13). Using a slightly different model, these authors discovered that extremely temperature sensitive areas existed in brain such as the caudatoputamen, while the lateral reticular nucleus of the thalamus was less temperature sensitive (12). A large number of investigations have demonstrated that intraischemic hypothermia, i.e. hypothermia introduced and maintained during the ischemic insult, diminishes neuronal damage and improves recovery following transient global (17, 20, 29, 32, 33, 35) and focal (16, 18, 19, 21, 22, 24, 25, 27, 28, 88) ischemia as well as trauma (89). Colbourne and Corbett (42) employed a long-term recording system for brain temperature in a gerbil model with a transient occlusion of the common carotid arteries for 5 min, which produced delayed neuronal death in hippocampal CA1 four days after the operation. These authors also established that a postischemic hypothermia introduced even as late as 4 hours after reperfusion at 34°C and maintained for 24 hours, could resuscitate CA1 neurons for at least 180 days of survival (43). This finding confirmed that at least in these animal models, postischemic mild hypothermia could resuscitate neurons that would otherwise die, in a delayed fashion for considerably long time (Table 1). These may encourage colleagues in the clinical field who are interested in the introduction of hypothermia. A suggestion that hypothermia could only shift the time of death of neurons to some days afterwards, was based on a rather special case of a duration of hypothermia being too short (20). Permanent ischemia models on the rat have also been employed for the test of hypothermia. The outcome of hypothermia was partly positive, while other tests were not significantly different from non-ischemic controls (15, 18, 36, 39, 44); these are summarized in Table 2.

MODE OF ACTION OF HYPOTHERMIA

• As was shown in hypothermic animals or animals in hibernation, metabolic rate was remarkably reduced at lower ed temperature (62, 67, 90, 91); this may explain the incre ased resistance of ischemic neurons to damage at lowered temperature. However, the degree of reduced oxygen and glucose demand at lowered temperature should always over come the degree of shortness of demand of these substances. There are many modes of ischemic neuronal death, and many details are still unknown (Fig. 1). At the very initial stage of ischemia, excitotoxicity plays a pivotal role (92, 93), where a large-scale glutamate surge (94) due to dysfunction of glutamate transporters is induced within 20-30 seconds after the start of ischemic insult in hippocampal CA1 of the gerbil (33). The extracellular glutamate level sometimes exceeds 20 and 100 times the basal levels, at 5 and 10 minutes after ischemia, respectively (95). In response to the glutamate surge, an intracellular calcium mobilization follows within 2-3 min after ischemic insults (96, 97). This calcium mobilization is lethal when it is sustained and exceeds beyond certain levels (98, 99), because calcium damages mitochondrial respiration and induces calcium dependent cascade reactions in an uncontrolled manner. When ischemic insults were performed at lowered temperature, the degree of glutamate surge is largely depressed (33, 100) and the onset of calcium mobilization is significantly delayed (101). In the delayed death model of the gerbil hippocampal CA1, neurons are resuscitated in a half of the animals at 35°C, and totally resuscitated at 33°C (33). However, in these gerbil models, excitotoxicity will entirely finish within one hour after the ischemic insults, while postischemic hypothermia introduced some hours after the insults still resuscitates hippocampal neurons as effectively as prior hypothermia (Fig. 2) (37, 41-43, 45). This finding indicates that temperature dependent cell processes are still going on after excitotoxicity. Here we should pay much attention to post-excitotoxic connections. One such connection should be the postischemic sustained activation of glutamate receptors. When hippocampal slices were subjected to a micro-fluorometry imaging for intracellular changes of calcium concentration, a mobilization of calcium in response to the addition of N-methyl-D-aspartate (NMDA), an agonist for glutamate receptors, was clearly demonstrated selectively in the strata radiatum and oriens of the ischemically vulnerable CA1 sector in an *in vitro* ischemic condition. In this model, the calcium responses to the NMDA addition were found to significantly increase in gerbils that had been subjected to transient ischemic insults for 5 min 1 to 9 hours, with a peak around 3-6 hours, before sacrifice (102). This apparently sustained activation of NMDA type glutamate receptors can be further confirmed by a patch clamp study on CA1 neurons in hippocampal slices of the gerbil (103). Thus, it was shown that practically no such sustained enhancements of the calcium mobilization could be detected in slices from gerbils that were subjected to the transient ischemia at lowered brain temperature around at 33°C (102).

Dramatic changes develop in ischemic neuron nuclei. It has been known that protein synthesis is almost totally and irreversibly depressed in the CA1 sector; that is a key mechanism of the delayed neuronal death (104). When the insults were performed at 30°C in a four-vessel-occlusion model of the rat, there could be seen an earlier recovery of the synthesis of protein along with neuronal survival in the CA1 (105, 106). Also, a marked enhancement of DNA binding ability of a transcription factor, activator protein-1 (AP-1), in gerbil hippocampus was found (107). In these cases, particularly in the

Table 1. Review of postischemic hypothermia for transient forebrain ischemia

Auther (Year)	Model	Hypothermia			Survival time	Result
		Start	Duration	Temperature		
Busto (1989)	Rat 10 min BCAO+hypothension	5 min 30 min	3 h	30℃	3 days	5 min : effective 30 min : non-effective
Buchan (1990)	Gerbil 5 min BCAO	immediately	8 h	34.5℃	5 days	effective
Coimbra (1990)	Gerbil 5 min BCAO	immediately	5 h	29℃	7 days	effective
Chopp (1991)	Rat 8 or 12 min BCAO+hypothension	immediately	2 h	30℃	7 days	8 min ischemia : effective 12 min ischemia : non-effective
Welsh (1991)	Gerbil 5 min BCAO	immediately	1, 2 h	23, 33℃	7 days	non-effective
Coimbra (1992)	Rat 10 min BCAO+hypothension	2 h	5 h	33℃	7 days	effective
Carroll (1992)	Gerbil 5 min BCAO	immediately ~ 3 h	30 min ~ 6 h	28 ~ 32℃	4 days	S;immediately, D≧ 2 h : effective S≦1 h, D≧6 h : effective
Dietrich (1993)	Rat 10 min BCAO+hypothension	immediately	3 h	30℃	3, 7, 60 days	3, 7 days: effective 60 days: non-effective
Coimbra (1994)	Rat 10 min BCAO+hypothension	2 ~ 36 h	5 h	33℃	7 days	2, 6 h : effective
Colbourne (1994)	Gerbil 3 or 5 min BCAO	1 h	12, 24 h	32℃	10, 30 days	effective
Colbourne (1995)	Gerbil 5 min BCAO	1, 4 h	24 h	32, 34℃	180 days	effective

BCAO ; bilateral carotid artery occlusion, S; start of hypothermia, D; duration of hypothermia

Table 2. Review of postischemic hypothermia for focal cerebral ischemia

Auther (year)	Model	Hypothermia			Survival time	Result
		Start	Duration	Temperature		
Onesti (1991)	Rat permanent MCAO	immediately	1 h	24℃	1 day	effective
Baker (1992)	Rat permanent MCAO	immediately ~ 3 h	1 h	24℃	1, 3 days	S≦1 h : effective S>1 h : non-effective
Moyer (1992)	Rat permanent MCAO	immediately 40 min	1 hr	32℃	1 day	S; immediately : effective S; 40 min : non-effective
Kader (1992)	Rat permanent MCAO	1 h	1 hr	33℃	1 day	effective
Morikawa (1992)	Rat permanent MCAO	immediately	2, 4 h	30℃	3 days	non-effective
Xue (1992)	Rat 3 h MCAO	immediately ~ 3 h	1.5 ~ 3 h	32℃	3 days	effective
Karibe (1994)	Rat 2 h MCAO	10 min ~ 1 h	1 ~ 2 h	32-33℃	1 day	S≦30 min : effective S; 1 h, D; 1 h : non-effective
Markarian (1996)	Rat 3 h MCAO	immediately ~ 45 min	1 ~ 4 h	32-33℃	3 days	S≦30 min : effective
Yanamoto (1996)	Rat 3 h MCAO	3, 3.5 h	1 ~ 20.5 h	D≦1 h : 32 ~ 33℃ D>1 h : 34 ~ 36℃	1, 2 day	S; 3 h, D; 1 h : non-effective S; 3 h, D; 21 h : effective S; 3.5 h, D; 20.5 h : non-effective

MCAO : middle cerebral artery occlusion, S : start of hypothermia, D : duration of hypothermia

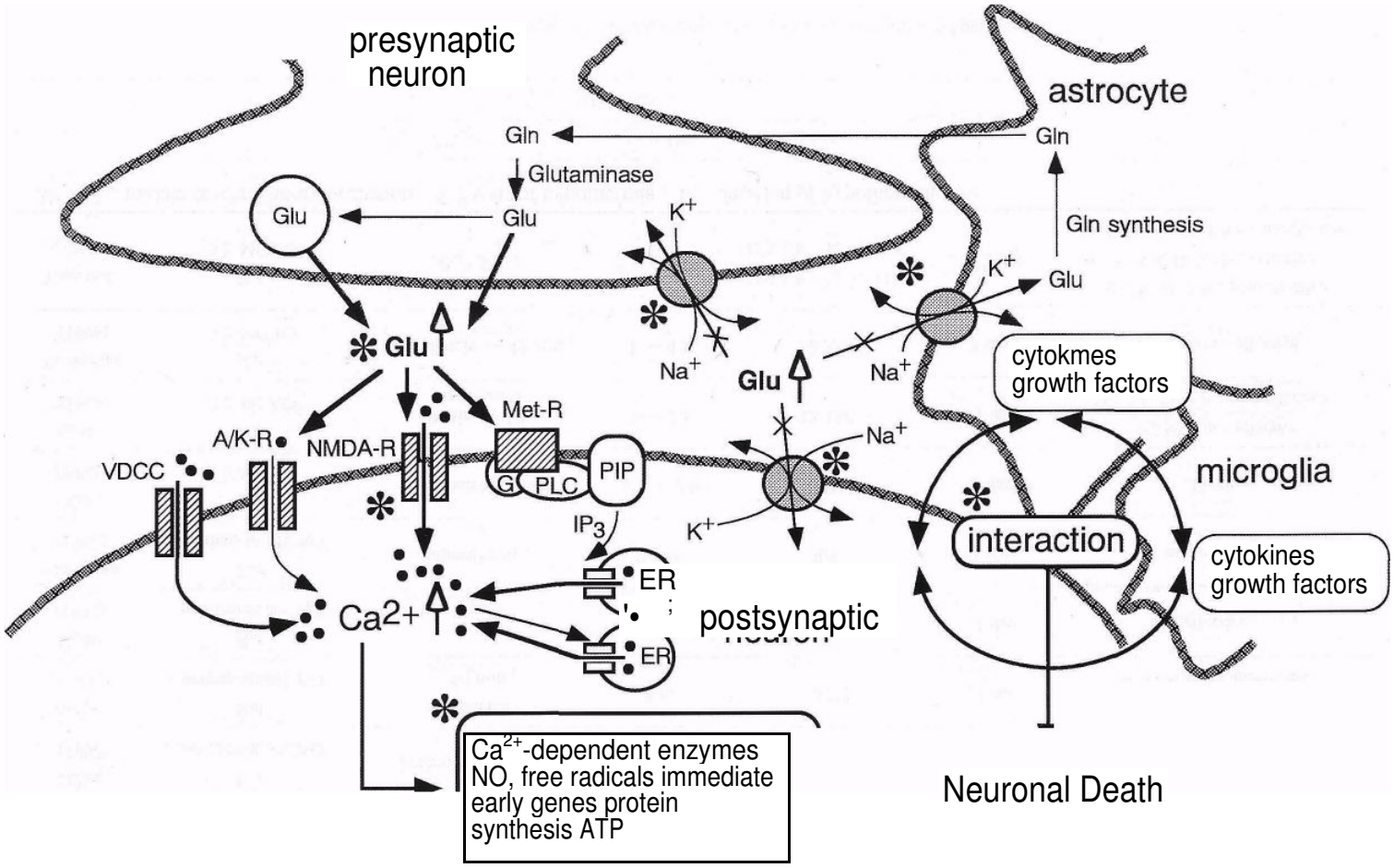

Figure 1. *Illustration of ischemic neuronal death. When the brain is exposed to ischemia, an immediate glutamate release occurs from presynaptic neurons followed by postsynaptic neurons and then glial cells. The extracellular glutamate surge induces a marked Ca^{2+} mobilization in neurons via glutamate receptors, which induces various Ca^{2+}-dependent cascade reactions. Not only neurons, but also astrocytes and microglial cells are profoundly affected by ischemic insult. Cerebral protection by hypothermia might be based on its non-specific widely progressing reactions in the whole ischemic entity shown in this figure (*).Glu, glutamate; Gin, glutamine; VDCC, voltage dependent Ca^{2+} channel; A/K-R, AMPA-kainate type glutamate receptor; NMDA-R, NMDA type glutamate receptor; Met-R, metabotropic glutamate receptor; G, G-protein; PLC, phospholipase C; PIP, phosphatidylinositol phosphate; IP_v inositol 1,4,5-trisphosphate; ER, endoplasmic reticulum.*

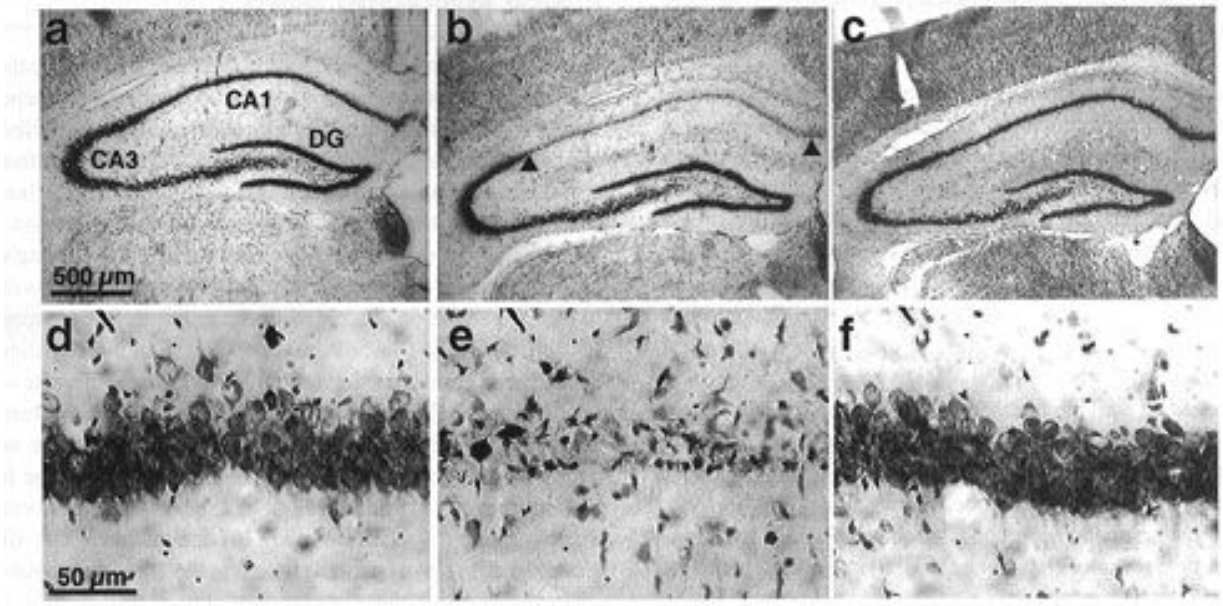

Figure 2. Photomicrographs of sections of gerbil hippocampus. Animals were sacrificed after 30 days of survival following 5 min of bilateral carotid artery occlusion, (a) and (d)are non-ischemic animals. In normothermic animals (b, e), there is a widespread neuronal destruction in CA1 sector, while almost all neurons in CA3 and dentate gyrus are preserved. In postischemically hypothermic animals (c, f) (hypothermia was initiated 1 h after the ischemic insult and maintained for 24 h), most neurons in CA1 sector are preserved, d, e, and f are higher magnifications of a portion ofCAl sector. Bars, 500 um (a, b, c), and 50 um (d, e, f) .

hippocampus, there were significant differences among the CA1, CA3 and dentate gyrus, including CA4, in terms of temporal profiles of the DNA binding activities; e.g. the time courses for the activity was prolonged from 1 to 9 hours in the CA3 and the dentate gyrus, while a rather short-lived enhancement was observed in the CA1, the area vulnerable to ischemia (108). However, gerbils with prior ischemic insults at 32°C, showed a prolonged period of binding activation, becoming closer to the profiles for ischemia-resistant CA3 and dentate gyrus. Similar prolongation can be seen in gerbils which are rendered resistant to ischemic insult by a prior loading with a short ischemic insult for 2 min (109). Although the protein which will be synthesized after AP-1 binds to an upstream region of the corresponding gene is still unidentified, it is not likely linked to protection resuscitation of deteriorating neurons.

In the rat middle cerebral artery occlusion model, a postischemically induced hypoperfusion followed by a hyperper-fusion disappeared when the operations were carried out in hypothermic conditions (110). Cerebral edema and failure of the blood-brain barrier, which are both pathophysiological events resulting from ischemia, can also be depressed by hypothermia. Although the knowledge of the cellular processes after ischemia is still only fragmentary , particularly of late phenomena after excitotoxicity, it is very likely that there is a multiplicity of cascade reactions which proceed both in temporal and spatial ranges. Endothelial cells as well as astroglial and microglial cells participate in the ischemic processes. In this relation, we have recently found an involvement of albumin in the ischemic response of microglial cells. When cultured microglial cells were exposed to a minute concentration of albumin, the cells remarkably proliferated, and significantly increased both phorbol ester-induced production of superoxide anions and lipopolysaccharide-induced formation of nitric oxide (111). It is known, that superoxide anions themselves and the product of their reaction with nitric oxide are harmful to neurons. In effect, it may be possible that, when albumin leaks out through the postischemically deteriorated blood-brain barrier, it starts to stimulate microglial cells to proliferate, which then induces production of super-oxide as well as nitric oxide by these cells, causing damage to ischemic neurons. Proliferating microglial cells could be seen in the immediate proximity of neurons shortly after onset of ischemic insult (112, 113). These findings suggest that microglial cells in ischemic conditions play a role as a damage inducer to neurons, although they behave as scavengers after neurons die. However, postischemic hypothermia depresses microglial proliferation (unpublished data). It is intriguing that cultured microglial cells produce less super-

oxide anions and nitric oxide at a temperature lower than 37°C (114).

Elucidation of mechanism of ischemic neuronal damage should lead to the development of drugs based on blocking or modifying these cellular processes. A variety of chemicals have been proposed in this regard so far for the possible clinical treatment of ischemic neuronal damage in stroke, injury, and transient cardiopulmonary arrest. Studies on excitotoxicity (92, 93, 115, 116) showed that the extracellu lar glutamate surge following ischemic insult might activate the receptors strongly and sustainedly. Molecular biological approaches have indicated that NMDA type glutamate recep tors are, when activated in ischemic condition, highly at risk, since the receptor-associated channels open for not only monovalent cations but also for calcium ions, which induces a deadly mobilization of intracellular calcium (2, 3, 98, 99). Although glutamate antagonists of both NMDA and non-NMDA type receptors have been studied in the CA1 sector of the hippocampus because of its extraordinary vulnerability to ischemic insults, a non-competitive antagonist of NMDA receptors, (+) -5-methyl-10, 1 l-dihydro-5H-dibenzo [a, d] cycloheptene-5, 10-imine maleate (MK-801) has attracted attention for its strong protective effect against ischemic damage of CA1 neurons at low doses (117, 118). However, later, the beneficial effect of MK-801was disputed as it was proved that the apparent neuroprotective action of MK-801 was not through its pharmacological properties, but through its temperature-lowering activity (30, 119, 120). Therefore, there has been a considerable controversy on whether MK-801 actually protects ischemic neurons, and the notion has been raised that studies on small animals to evaluate any chemical for its neuroprotective potential should include analysis for its effect on brain temperature when adminis tered. As far as we were aware of this, a large number of chemicals reported to be neuroprotective have been found to be much less active than described, even inactive, when re-evaluated in our experimental paradigm. Thus, we have de signed a telemeter-based brain temperature control system that allows continuous monitoring and regulation of brain temperature at any selected degree in conscious and freely moving animals (121). Using this instrument, we can control brain temperature in a narrow range around at 37°C, and observed a selective, delayed neuronal death in hippocampal CA1 of gerbils that were subjected to a global cerebral is chemia for 5 min. The following outcomes were then ob tained: CA1 neurons could certainly survive from ischemic damage on the intraperitoneal administration of MK-801 only at doses of more than 10 mg/kg, much higher than those employed in the previous studies, which, showing similar survival on 1-3 mg/kg, had no brain temperature control at all (120).

CLINICAL HYPOTHERMIA UPDATE

• Three randomized control studies of mild hypother mia in patients with severe closed-head injury were indepen dently reported in 1993. They all revealed beneficial effects. When hypothermia was introduced at 32°C to 33°C within a mean of 10 hours after injury and maintained for 24 hours followed by a slow rewarming, a significant reduction of intracranial pressure as well as cerebral blood flow during the cooling period was obtained without any rebound (49). Twelve of the 20 patients in the hypothermia group showed outcome of moderate, mild or no disabilities, while 8 of the 20 patients did in the normothermia group (49). When hypothermia was introduced at 34°C for 2 days followed by a slow rewarming, a significant reduction of cerebral perfusion pressure was demonstrated (50). Eight of 16 patients survived in the hy pothermia group, while 3 of 17 patients survived in the normo thermia group (50). Clifton *et al* (46) demonstrated that after 3 months, the ratio of patients with less than moderate disabil ity to patients with more severe disability was 12 to 11 in the group where hypothermia was introduced within 6 hours at 32-33°C for 2 days followed by a slow rewarming, and 8 to 14 in the normothermia group.

Recently in Japan, a research project chaired by one of the authors (K.K.), entitled "Mild hypothermia for a treatment of stroke - basic investigations and clinical studies" has been organized in the 1994-1996 fiscal year under the support of the Ministry of Health and Welfare. Although studies should be extended further, the project made the following contemporary consensus at the conclusion of the project (56): hypothermia can be applied to patients with brain injury, cerebral infarction, subarachnoidal bleeding (after clipping) and transient cardiopulmonary arrest in their very acute stage. Basic intensive control of general and cerebral circulation with sufficient oxygen supply should be secured. For anesthesia, the use of midazolam (GABA agonist) or droperidol (a-adrenergic blocker) is recommended. Systemic blood pressure, cerebral perfusion pressure and oxygen supply should be more than 100 mmHg, 80 mmHg and 800 ml/min, respectively. Oxygen consumption rate and intracranial pressure should be less than 25% and 20 mmHg, respectively. Brain temperature could be kept at 33-34°C, while 35°C is recommended for less experienced institutions. The use of a blanket for surface cooling, with occasional intragastric cooling, is suggested. Intracerebral temperature is to be monitored by intrajugular temperature measurement, with occasional monitoring of the temperature of the tympanic membrane, urinary bladder or pulmonary vein. The period of hypothermia is not to be limited. However, occasional severe infections should be carefully and intensively controlled. Rewarming should he slowly performed, for instance at the rate of 0.1 °C per hour with a sustaining period at 35°C. Cardiac output and the oxygen

saturation ratio of mixed venous blood should be monitored with a Swan-Ganz catheter. Platelet transfusion and suplementation of potassium may be occasionally needed, and hemoglobin content in blood stream should be kept at greater than 12 g/dl. In addition to these clinical suggestions, the project emphasized the need to organize large-scale randomized studies involving different institutions exchanging information and ideas, in the overall prospects for future improvement of hypothermia treatment.

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