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Heat-induced Neuronal Injury: A Review of Cellular and Animal Models

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The increasing incidence and lack of effective therapeutic approaches for heat-related illnesses have attracted much research attention. Hyperthermia occurs along a continuum of heat-related conditions, starting with heat stress, progressing to heat exhaustion, heat stroke and culminating in multiorgan dysfunction and death in some instances. Neuronal injury is a major concern to clinicians because of the permanent neurological sequelae in patients who survive heat illness. Despite extensive studies in this field, the mechanisms underlying heat-induced neuronal injuries remain unclear. Many experimental models using animals or cell cultures exposed to heating conditions have been employed to study heat-induced neuronal injury. The heating conditions vary with intensity, duration of exposure and application of anesthetics, leading to variations in stress responses. In this review we extend from clinical findings to animal and cell culture models in which neurons have been injured by heat exposure. We compare the advantages and disadvantages of these models and propose a possible mechanism for the pathogenesis of heat-induced neuronal injury.

Key words: Neuronal Injury, Hyperthermia, Heat Exposure, Animal Models, Cell Cultures

CONTENTS

INTRODUCTION

Rigorous exercises or doing strenuous work in hot environments often leads to heat-related illness. Thus, military personnel going through heavy training are prone to suffer from such illness. With the problems of global warming, the increase in heat-related illnesses has attracted attention worldwide[1,2]. Heat-induced illness is typically depicted as a series of discrete events from innocuous conditions (heat cramps) through moderate (heat exhaustion) to the most serious (heatstroke)[3-5]. Clinical heatstroke symptoms often consist of hyperthermia, mental confusion and hot and dry skin. Serious complications can include brain damage, respiratory and cardiovascular collapse and renal failure. The mortality and morbidity in heatstroke victims remains high, despite intensive therapeutic interventions[6]. Heat illnesses have attracted much

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Table 1. Clinical evidence of neuronal damage in heatstroke patients

Reference	Heatstroke form	Age (yr)/sex	Body temperature (°C)	Predisposing factor	Neurologic complication	Systemic complication	Clinical manipulation	Duration of hosp. (day)	Brain damage	Outcome
Mahajan and Schucany 2008[91]	Classic	54/M	42.2	Cocaine abuse, skin graft infection	Altered mental status	Hypotension, respiratory failure	Not known	＞30	Bilateral cerebellar encephalomalacia	Permanent aphasia
Jafferany and Lowry 2008[92]	Exertional	16/M	41.1	None	Insomnia, intermittently agitated with significant disorientation, confusion, delirium and hallucination	Erythematous maculopapular rash, respiratory failure, severe rhabdomyolysis, renal failure, hepatic dysfunction, and disseminated intravascular coagulation	Intubation, rehydration, cooling, hemodialysis, olanzapine 2.5 mg orally	29	None	Survival; no sequelae
Rav-Acha et al. 2007[93]	Exertional	30/M	40	Fatigue, diarrhea	Disorientation, convulsion and unconsciousness	Disseminated intravascular coagulation, severe rhabdomyolysis, renal failure, hepatic dysfunction	Intubation, rehydration, cooling, hemodialysis	35	Persistent subcortical dysfunction	Slight motor disinhibition, slowed speech and impaired writing
Aibiki et al. 2005[94]	Exertional	34/M	43.5	Not known	Unconsciousness	Disseminated intravascular coagulation, acute renal failure, high c-reactive protein level, subsequent disseminated fungal infection	Intubation, rehydration, cooling, administration of buprenorhine, diazepam and vecuronium	14	Cerebellar infarction, and bilateral fungal oculitis	Ataxia
Albukrek et al. 1997[95]	Exertional	45/M	42	Not known	Mild depression, convulsion and coma	Disseminated intravascular coagulation, mild rhabdomyolysis	Intubation, cooling, intravenous diazepam (5 mg)	21	Crebellum atrophy	Ataxia, slurred and intelligible speech
Ohshima et al. 1992[96]	Classic	52-day-old/ F	41.3	None	Unconscious and pupils dilation	No response to vasopressor drugs	Cardiac massage and oxygen inhalation	Reach hospital dead	Brain edema and flat gyri	Death
Delgado et al. 1985[97]	Classic	66/M	42.5	Prior medication: amitryptiline (30 mg/day), imipramine (75 mg/day) and pherphenazine (6 mg/day)	Manic depressive psychosis, moderate drowsiness and mental confusion, absence of muscle stretch and skin reflexes	Acute respiratory insufficiency, rhabdomyolysis	Intubation, cooling	14	Almost total loss of Purkinje cells, with proliferation of Bergmann glia, necrosis of the granular layer , dentate nucleus neuronal loss, chromatolysis and neuronophagia in anterior and intermediolateral horns of spinal cord, macrophages phagocytosing myelin in the anterior roots	Death

attention, however the mechanisms underlying heat-induced neuronal injuries remain unclear[7]. There are several established animal models that produce hyperthermia leading to pathological changes similar to those observed clinically in affected patients (Table 1). Depending on whether anesthetics are applied, animal models can be divided into two categories: passive and active heating. Both can produce systemic responses. The anesthetized animals' rectal temperature can be maintained at the exposure temperature for desired periods to induce neuronal

damage without causing death[8,9]. Conversely, in active heating models that do not use anesthetics, the variations in individual stress responses can be difficult to prevent. Nevertheless, active heating models can reveal normal physiological responses that are hidden in anesthetized animals so that alterations in brain function can be reported[10,11]. In addition to whole body heating (WBH), regional heating is widely used in thermal therapy for tumors and can become a model for thermal toxicity research on specific regions or organs of interest. Neuronal culture can also be used to study the direct effect of heat on neuronal injuries. However, cell culture studies are devoid of systemic modulation, making interpretation of the results limited. Therefore, cellular and animal models possess distinct advantages and disadvantages and need to be compared carefully.

CLINICAL EVIDENCE OF HYPERTHERMIA-INDUCED BRAIN INJURY

High temperature exposure for prolonged periods or strenuous physical exercise in hot environments can lead to hyperthermia, when the heat dissipation mechanisms are compromised and body temperature rises above the hypothalamic thermoregulation set point. If sustained, hyperthermia may produce acute physiological alterations (e.g., circulatory failure, hypoxia and increased metabolic demand) and to inflammatory and coagulatory responses[12-19]. The interplay among these events leads to alterations in blood microcirculation and results in injury to the vascular endothelium and tissues as seen in patients with heatstroke[15, 20-23]. Even with adequate lowering of the body temperature and aggressive treatment, heat stroke is often fatal and those who do survive can sustain permanent neurological damage[5,24]. Certain drugs can induce or exacerbate hyperthermia-induced brain damage, such as the tricyclic antidepressants, antihistamines, amphetamines and diuretics[25,26]. However, it is unclear whether age, sex, drug abuse, alcoholism or acute and chronic diseases such as diabetes, cardiovascular, endocrine or metabolic ailments, depression or dementia further influence the vulnerability of humans to heat-related death[7].

Table 1 documents the forms of heatstroke among patients experiencing hyperthermia, producing neurological and systemic complications and ultimately resulting in death, or in survival with varying degrees of neurological deficits. The literature on heat-related illnesses in humans is very limited and only a few observations on the brain pathologies associated with heatstroke have been documented. Patients diagnosed with heatstroke can die at different time intervals ranging from 6 h to several weeks after heat illness, and they exhibit profound pathological changes in the cerebellum and cerebral cortex[27].

Brahams has proposed a paradigm of the affected brain regions in heat-related illnesses as follows[28]. In the cerebellum, the Purkinje cell layer exhibits considerable loss of cells and prominent edema. However, the molecular and granular layers in the cerebellum are usually normal. The dentate nucleus of the cerebellum is the most sensitive structure and shows signs of degeneration of nerve cells and hyperchromatic reactions. Damage to the cerebral cortex in heat-injured victims includes glial cell proliferation and activation of microglia. The white matter of the cortex is tolerant to heat with no detectable signs of demyelination or vesiculation of the myelin. In general, glial cell reactions are more prominent in the Bergmann layer, followed by the molecular layer. In the hypothalamus, the hypothalamic nuclei are injured mildly but with visible loss of neurons and a slight increase in the number of glial cells in some edematous regions. In the brain stem or other parts of the brain, there are no noticeable cell changes or edema. Microhemorrhages of the perivascular space in leptomeninges are common. Brain regions with the most pronounced hemorrhage are the paraventricular nucleus, the supraoptic nucleus, the medial parts of the ventromedial and the dorsomedial hypothalamic nuclei. There are either no hemorrhages or mild forms in the perifornical and septal regions and the medial portion of the thalamus. The pons and medulla oblongata exhibit local hemorrhages, which are largely confined to the floor of the fourth ventricle and/or near the dorsal efferent nucleus of the vagus.

NEURONAL INJURY FOLLOWING WBH IN ANIMAL MODELS

Given the physiological and pathophysiological alterations in heat-related illnesses, WBH has been established as a model for research. Based on experience of human patients subjected to cancer thermal therapy, passive heating models in which animals have the whole body heated under anesthesia have been applied. In contrast to passive heating models, an active heating type without the application of anesthetics has also been established. Differences between these models include the application of anesthetics, the exposed temperature, the duration of heating and various physiological responses to heat that might be compromised by anesthetics. The common methods of WBH used to produce hyperthermia in animals with and without anesthesia are listed in Table 2. The acute phase response to heat involves endothelial cells, leukocyte re-

Table2. The evidence of neuronal injuries following whole body heating in animal models

References	Species	Anesthesia	Heating method	Heat exposure	Survival time	Hemodynamic and metabolic changes	Molecular and cellular changes in brain
Cheng et al., 2007[89]	Rat	Urethane anesthesia	Heating pad	43℃ for ~63mins	~25 mins	Arterial hypotension, intracranial hypertension, cerebral ischemia and increased levels of NO_2^-, glutamate, glycerol, and lactate/pyruvate ratio in hypothalamus	Nerve cell shrinkage, pyknosis of the nucleus, and loss of Nissl substance in hypothalamus and increased nNOS expression
Chou et al., 2005[98]	Rabbit	Urethane anesthesia	Heating blanket	45℃ for ~115mins	~40 mins	Hypotension	Dopamine overload in the striatum
Yang and Lin, 2002[38]	Rat	Urethane anesthesia	Hot air	42℃ for ~87mins	~25 mins	Arterial hypotension, intracranial hypertension, cerebral ischemia, increased levels of dihydroxybenzoic acid in the striatum, increased $O_2 \cdot^-$ generation in the brain, liver, and heart	Neuronal damage and lipid peroxidation in cortex, hippocampus, striatum and hypothalamus
Bouchama et al., 2005[99]	Baboon	Ketamine and diazepam anesthesia	Hot air	44-47℃ until core temp.＞ 43℃	~3 hrs (replaced with saline and dextrose)	Increased plasma IL-6, prothrombin time, activated partial thromboplastin time, and D-dimer levels, and decreased platelet count, increased plasma levels of thrombomodulin, creatinine, creatine kinase, lactic dehydrogenase, and alanine aminotransferase	Neuronal necrosis in pallidum, cerebellar Purkinje cells necrosis
Sharma, 2007[29]; Sharma and Johanson, 2007[100]	Rat	Conscious	Hot air	38℃ for 4 hrs	Seldom death	Occurrence of salivation, prostration and gastric ulcerations, mild hypotention	Choroidal epithelium degeneration, dark and distorted neurons (some with eccentric nucleolus) in edematous regions of the cerebral cortex, hippocampus, brain stem, cerebellum, thalamus and hypothalamus along with severe loss of nerve cells, marked neuronal damage in the CA-4 subfield of hippocampus, swollen synapses with damage to both pre- and post-synaptic membranes commonly seen in many brain regions, profound upregulation of glial fibrillary acidic protein in the brain stem, cerebellum, thalamus and hypothalamus, vesiculation of myelin and swollen axons commonly found at the ultrastructural level

sponses and epithelial cells. These cells protect the body against tissue injury and will promote prompt repair in the event of injury. The pathophysiological sequence of events in heatstroke is similar to that found in severe sepsis.

(A)Animal Models with Passive Heating under Anesthesia

Use of Heating Chambers to Induce Hyperthermia

Anesthetized animals can be kept in a laboratory incubator with free airflow facilities at temperatures of 40-45 °C for short durations (30 min to 1 h). In anesthetized rats or mice the rises in body temperature are gradual and may reach to the level of the exposure temperature within 1 h. When the body temperature reaches 42 °C, this can be maintained for 30 min or 1 h by adjusting the exposure temperature[29]. Further prolongation of heat exposure will result in death of the animals because of excessive heat-induced membrane and enzymatic damage. Care should be taken to avoid increases in the body temperature beyond 42 °C. Under anesthesia, thermoregulation is normally absent. Therefore, the exposed anesthetized animal can reach a preset temperature that can be maintained for a long time[30,31]. In this model, excessively high exposure temperatures (40-60 °C) are employed for effectively inducing neuronal damage, and this resulted in the death of many animals[32, 33]. In general, this animal model of hyperthermia is not comparable with the local environmental situations to which human populations are normally exposed[34,35].

Use of Heating Pads to Induce Hyperthermia

This model has been used to induce heatstroke under anesthesia in rats and can be extended to other species. Before the induction of heatstroke, the rat's core temperature is maintained at about 36 °C with a folded heating pad

to maintain the baseline temperature. During the experiment, the ambient relative humidity should be controlled at about 60%. Heatstroke is induced by increasing the temperature of the folded heating pad to 43 °C with circulating hot water. The moment at which the mean arterial pressure drops by a value of 25 mmHg from its peak levels is taken as a reference point for the onset of heatstroke[36-38]. Immediately after the onset of heatstroke, the heating pad must be removed and the animal is allowed to recover at room temperature. All animals with heatstroke in these studies died during or shortly after heating. The survival time should be recorded to compare the pharmacological or other treatment effectiveness used in each designed group.

(B) Animal Models Using Active Heating without Anesthesia

It is important to note that most of the anesthetics applied in heat stress investigations, such as pentobarbital and ketamine, are neuroprotective agents following various forms of ischemia or metabolic insults to the nervous system[39]. Sharma and colleagues developed a rat model without the use of anesthetics that produced mild hyperthermia not leading to heat stroke[29]. In this model, rats were exposed to WBH at 38 °C in a biological oxygen demand incubator for 4 h. The relative humidity (45-50%) and airflow velocity (20-25 cm/sec) were kept constant. At the end of 4 h WBH at 38 °C, some rats lay prone in their cages and did not move even after gentle pushing although their righting reflex was not lost. A few rats (<20%) whose body temperature exceeded 42 °C died either during, or a few minutes after termination of WBH. This model has been employed to study alterations in blood brain barrier (BBB) permeability and the ionic, chemical and immunological microenvironments involved in the development of brain pathology during heat stress[14,40-43].

NEURONAL INJURY IN LOCAL HEATING MODELS

Regional or local heating has been employed to kill cancer cells, and associated neuronal injuries caused by local heating have been demonstrated[44,45]. A local increase in tissue temperature is one of the primary causes of abnormal cell reactions in the brain during heat exposure, but very little is known about the effect of local heating on the human central nervous system (CNS). In patients, no brain damage developed when an antenna at a temperature of up to 45 °C was placed on tumors. However, heating of peritumoral brain regions showed cell injury[46]. When the temperature of the heated tumor tissue reached 44-49 °C, aggravation of the peritumoral edema and focal brain swelling were observed[45]. In contrast to this study, deep brain heating in many patients did not result in neurotoxicity[44]. Experimental evidence in animal models showed that local brain heating was associated with edema formation, hemorrhage, necrosis, inflammation, gliosis and neuronal damage[47]. In these experiments, death after hyperthermic insults to the brain was very common. However, as no death was observed in experiments on cats up to 56 days after heat treatment, it was concluded that heating of a small volume of the brain did not alter other vital functions[48,49]. Local heating of the spinal cord in rats or mice resulted in paralysis associated with neuronal injury[47,50]. However, the degree of injury varied in different brain regions induced by local heating, so this needs further investigation to elucidate the underlying mechanisms.

HEAT-INDUCED NEURONAL INJURY IN CELL CULTURE MODELS

It is interesting to elucidate what temperature level will have a direct destructive action on cells. Although heat is a factor inducing or potentiating neuronal damage, it can also be adaptive within some limits. Thus, it is difficult to draw the line between physiological and pathological levels of heat exposure. The degree of cellular damage depends on both the duration and intensity of the heating. Increases in the duration of heating caused cellular damage for many cell types in an approximately logarithmic manner within a range of 42-50 °C[51]. Exposure of cultured neurons to 43 °C for 1.5-2 h resulted in cell death, whereas at a higher temperature (45 °C), a shorter duration (30 min) was lethal[52,53]. In primary cultured striatal neurons stressed for 2 h at 43 °C, the first sign of damage was blebbing of processes, visualized microscopically ~24 h after the termination of stress. The appearance of condensed nuclei was seen in apoptotic or necrotic cells within a period of 24-36 h[52]. Similar pathological patterns caused by heat exposure could be visualized in cortical neurons[53], cerebellar granular layer neurons[54] and neuroblastoma cells[54, 55]. If there is a clear-cut critical point, it might be at 40.0 °C. In neuronal cells, the cellular elements most sensitive to temperature were the mitochondrial and plasma membranes, in which irreversible transitions in protein structure or arrangements began to occur at temperatures higher than 40 °C[56-58]. Thus, 40.0 °C may be considered to be the critical thermal maximum.

ADVANTAGES AND DISADVANTAGES IN CELLULAR AND ANIMAL HEATING MODELS

The clinical and pathological findings for each cell type of the brain in response to heat can be represented readily in cell culture models. Cellular alterations in functions such as signal transduction and gene expression can be confirmed. Constancy in the temperature of the cell population can be more precisely controlled than in manipulation of the whole animal. However, cultured cells are devoid of systemic and regional contributions from surrounding cells. Local heating causes significant neuronal injuries without animal death when the desired parts of the CNS are exposed to high temperatures for an appropriate period. It has been postulated that local heat-induced damages are confined to areas the heat actually reaches[45]. These must be caused by the direct effect of heat, as this model did not induce a systemic response. Thus, such a local heating model might be suitable for studying the direct effect of heating on neuronal injury. However, it is still controversial because local heating still induces immunomodulation[59].

In passive (anesthetized) heating models, the death rate is much lower than in active models and a fixed body temperature can be maintained according to the experimental protocol[29]. Moreover, repeated and long-term effects of heat exposure can be achieved without any further risk of death. However, the countering physiological mechanisms used to avoid heat injury are compromised in passive heating models. Thus, the body's responses to thermal stress as well as individual variations among different animals are difficult to assess. Therefore, the findings obtained from passive heating models should be compared carefully with other models that do not use anesthetics to understand the hyperthermic injury in nature. In some studies using passive heating models, animals were exposed to extremely high temperatures (41-44 ^{o}C) until they developed neurological symptoms of heatstroke. In these models the mortality was very high (more than 80%), thus the study of brain dysfunction in situ was not possible[60]. Active (unanesthetized) heating models are close to the clinical conditions of mild-to-moderate hyperthermia, representing "heat stress" and "heat exhaustion". The symptoms of heatstroke itself are largely absent in these models, which represent a physiological response of stress reaction to heat in animals. This is more obvious from the activation of the body's natural defense system, physiological response of heat dissipation and behavioral alterations. In addition, the exposure temperature is in the physiological range. Therefore heat-induced degeneration of thermoreceptors or burning of skin receptors is unlikely in such models[29]. In contrast, the degree of hyperthermia varies according to the individual response of animals. Thus, maintenance of a certain magnitude of temperature in individual animals in these models is not possible[29].

THE DEVELOPING AND IMMATURE NERVOUS SYSTEM IS PARTICULARLY VULNERABLE TO HYPERTHERMIA

In any investigation of heat-induced neuronal injury, the subject's age must be taken into account because vulnerable stages exist during development. The embryonic brain is damaged when exposed to temperatures of 41 ^{o}C or above[61]. In addition, developmental events such as neuronal migration and proliferation of neuronal progenitors are particularly sensitive to hyperthermia. For example, the death of guinea pig embryos subjected to heat in utero was associated with abnormal cardiac development because heat induced defects in the migration of neural crest cells[62]. Hinoue et al. found that just 12 min of exposure to 43 ^{o}C during embryonic development could produce neuronal apoptosis leading to reduced thickness of the cortical gray matter[63]. In guinea pigs, 1 h heat exposure during early neurogenesis increased the incidence of microencephaly[64]. Mouse embryos exposed to 42 ^{o}C for 12 min or to 43 ^{o}C for 7-10 min exhibited anterior neural tube defects[65]. Embryonic days 13 and 21 were two teratogenic windows in guinea pigs when exposed to high maternal body temperatures[66]. Milunsky et al. also found that exposure to hot tubs, saunas, or fever during the first trimester of human pregnancy was correlated with an increased risk of neural tube defects[67]. Fever increases apoptosis in dividing cells of the testis and thymus suggesting that actively dividing populations are highly susceptible to hyperthermia-induced apoptosis[68]. The stage of development remains an important factor influencing heat stress response even after birth, probably by desensitization of several neurochemical receptors in the CNS. The influence of age on heat-induced alterations was examined in adult rats, where heat-induced changes in the BBB permeability, brain edema and cell changes were considerably reduced compared with young rats[69]. These experimental data showed that the intensity of heating, its perception at the cellular level and elicitation of stress responses all decreased with advancing age. Another possibility is that the processing of thermoregulatory information in the CNS by thermoregulatory effectors is more efficient in adult animals when compared with the young.

POSSIBLE MECHANISMS UNDERLYING HEAT-INDUCED INJURY IN CULTURED NEURONS

It has been believed that heat leads to neuronal injury, as evidenced from experimental and clinical studies. However, its underlying mechanism still remains to be elucidated. From neuronal culture studies, exposure to 43 °C for 1.5-2 h could lead to a delay in cell death. Delayed caspase activation following heating in cultured neuroblastoma cells[55,70] and primary rat neurons[52] were responsible for this phenomenon. The delayed cell death could be attributed to the activation of upstream caspases rather than caspase-3 because a pan-caspase inhibitor produces longer survival of cultured neurons exposed to heat than a caspase-3 specific inhibitor. One of the upstream caspases, caspase-9, was cleaved and activated following heating[71]. However, caspase inhibitors did not permanently protect neurons exposed to heat. This suggests that other and/or caspase-independent pathways might also be involved in heat-induced death. Heat may also damage mitochondria directly as mitochondrial depolarization can be induced by heat exposure, leading to an increase in the permeability of the mitochondrial inner membrane[72,73]. Mitochondrial depolarization interferes with two important mitochondrial functions, ATP production and calcium sequestration, which produce delayed neuronal death. Moreover, several studies have detected an increase in cellular reactive oxygen species (ROS) during heat exposure[74-76]. The increased levels of ROS and other free radicals can induce DNA damage that leads to apoptosis. Others pathways involving MAP kinases such as SAPK2/p38 and JNK and ERK can be activated by heat exposure[77]. However, inhibitors of these kinases did not protect cultured neurons against heat-induced apoptosis[78]. Heating has also been shown to produce endoplasmic reticulum (ER) stress by denaturing newly synthesized proteins in some cell types[79]. Under conditions of ER stress, the transcriptional upregulation of heat shock proteins were triggered and these proteins could protect neurons from heat-induced damage[80,81].

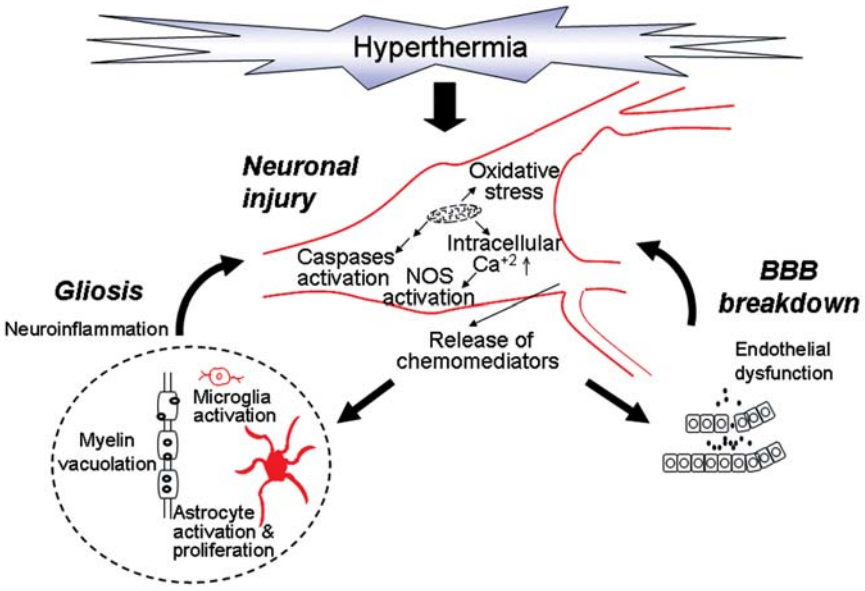

Fig. 1 Brain damage induced by hyperthermia. Membranes of neuron and its mitochondria are damaged during hyperthermia initiating multiple mechanisms such as increased levels of intracellular Ca^{+2}, caspases activation, oxidative stress and chemomediators secretion which may lead to BBB breakdown and gliosis. The gliosis and BBB disruption which also caused by hyperthermia may aggravate neuronal injury.

POSSIBLE MECHANISMS UNDERLYING BRAIN INJURY CAUSED BY WBH

Animals might be affected by heat exposure initially and then suffer from heatstroke if they are exposed to sustained heat. The following mechanisms have been proposed in the literature. Brain hyperthermia can induce a transient accumulation of prostaglandins, cAMP or cGMP in cerebral microvessels leading to an increase in vesicular transport of tracer substances[82]. Hyperthermia also stimulates nitric oxide (NO) production either directly or through cytokine release in the brain. Increased production of NO causes breakdown of the BBB permeability through cGMP-mediated mechanisms[83]. Taken together, the evidence suggests that hyperthermia increases prostaglandin and NO production and thus stimulates cAMP and/or cGMP synthesis in cerebral microvessels, causing breakdown of the BBB permeability. Leakage of plasma proteins across the microvessels following BBB disruption is also an important factor contributing to vasogenic brain edema formation and cell injury. In addition to brain edema, cutaneous vasodilation, splanchnic vasoconstriction, hyperpyrexia, hypotension and intracranial hypertension also can be observed in a heat stress state and is more serious at the onset of heatstroke. In animals suffering heatstroke, the hypotension and intracranial hypertension lead to cerebral ischemia and neuronal damage in the CNS caused by the

cessation of blood flow leading to oxygen and nutrient deprivation, and by enhanced secondary injury. This neurotoxic cascade involves overloading with ROS and reactive nitrogen species, overproduction of glutamate, dopamine and serotonin, cerebral inflammation and activated coagulation. It has been proposed that approaches that could attenuate the hypotension, hyperpyrexia, hypercoagulable state, inflammation, cerebral ischemia and metabolic acidosis might be used to alleviate neuronal injury[84].

CONCLUSION

Accumulated evidence demonstrates that neurons are vulnerable to heat exposure[24,85,86]. Neuronal culture incubation at 43-45 ^{o}C could cause apoptosis or necrosis[52,53,55]. Local and WBH with temperatures ranging from 38 to 43 ^{o}C for appropriate times lead to injuries in axons, neurons and glial cells[29,38,87-89]. Temperature elevations by themselves cause no damage but can become damaging in combination with other stresses involving disrupted energy metabolism (ischemia, hypoglycemia) or in developing or young stages in which the natural defense mechanisms against heat stress are compromised. The mechanisms underlying neuronal injury induced by heat exposure are illustrated in Fig. 1. The initial event might be mitochondrial depolarization induced by heating, leading to caspase activation[52,55,70], altered ATP production[72,73] and calcium sequestration[90]. Increases in intracellular levels of calcium might enhance the release of neurotransmitters such as glutamate or induce calcium dependent NO synthase activation leading to neurotoxicity. Moreover, ROS production is also induced by the mitochondrial failure caused by heat exposure[74-76]. These insults can induce the release of several neurochemicals and the generation of free radicals. The associated metabolic reactions result in profound alterations in the extracellular microenvironment, which can induce vasogenic edema and contribute to neuronal damage, depending on neuronal maturity, and on the degree and the duration of the temperature elevation. Further studies are needed to clarify these mechanisms. The models mentioned above have investigated mechanisms underlying heat-induced neuronal injury. Cell culture models may be easily repeated for studying signaling, gene expression and the assessment of neuroprotective agents. Local heating can be employed to study heat-induced injury in organs of interest. The active heating model prevents the compromised effects of anesthetics on stress responses. In addition, the exposure to temperature in this animal model is closer to actual hot weather situations than passive heating models, which need greater intensity and duration of heating because the normal physiological responses are compromised by anesthesia. Even though passive heating models have limitations because of the effects of anesthetics, they can produce neuronal injury more stably through prolonged heat exposure within a maintained range of temperatures. Taken together, these models can be employed selectively depending on experimental design. Studying the mechanisms of heat-induced neuronal damage will provide new insight for developing strategies for the treatment of heat-related neurological deficits.

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