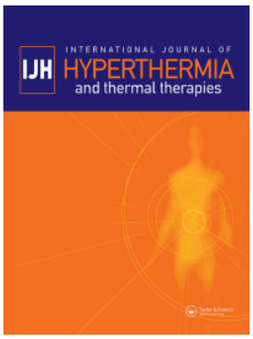


International Journal of Hyperthermia

ISSN: 0265-6736 (Print) 1464-5157 (Online) Journal homepage: informahealthcare.com/journals/ihyt20

Hyperthermia induced pathophysiology of the central nervous system

H. S. Sharma & P. J. Hoopes

To cite this article: H. S. Sharma & P. J. Hoopes (2003) Hyperthermia induced pathophysiology of the central nervous system, International Journal of Hyperthermia, 19:3, 325-354, DOI: 10.1080/0265673021000054621

To link to this article: https://doi.org/10.1080/0265673021000054621

INT. J. HYPERTHERMIA
VOL. 19, NO. 3 (MAY–JUNE 2003), pp. 325–354

Taylor & Francis
healthsciences

Hyperthermia induced pathophysiology of the central nervous system

H. S. SHARMA†* AND P. J. HOOPES‡

† Laboratory of Neuroanatomy, Department of Medical Cell Biology, Biomedical Centre, Uppsala University, SE-75123 Uppsala, Sweden
‡ Departments of Surgery and Radiation Oncology, Dartmouth Medical School and the Thayer School of Engineering, Dartmouth College Hanover, NH 03756, USA

(*Received 22 May 2002; revised 5 September 2002; accepted 14 October 2002*)

This review is focused on the pathophysiology of the central nervous system (CNS) associated with mild-to-moderate hyperthermia (body temperature $>37^{\circ}C$ but $<40^{\circ}C$) induced thermal stress in Human cases as well as whole body hyperthermia (WBH) in animal studies. Pathological changes can be observed in the nerve cells and glial cells in Humans following mild-to-moderate thermal exposure. On the other hand, morphological changes in the axons, nerve cells, glial cells and vascular endothelium is seen at the cellular and the molecular levels in rats subjected to heat exposure at $38^{\circ}C$ for 4 h (body temperature $>40^{\circ}C$ but $<42^{\circ}C$). This effect depends on the age of the animals and their prior thermal experiences. Taken together, heat stress induced hyperthermia, once believed to be non-toxic in the mammalian CNS, do produce specific alterations in the CNS that may have long-term behavioural, physiological and neuropathological consequences. The probable mechanism(s) underlying hyperthermia induced brain pathology is discussed.

Key words: Hyperthermia, CNS, oedema, blood–brain barrier, anaesthesia, repeated exposure, astrocytes, myelin, blood flow, serotonin, neuropathology, nitric oxide, carbon monoxide, GFAP, heat shock proteins, anaesthesia, ageing.

1. Introduction

Problems of hyperthermia and related brain dysfunction is known to human beings during early periods of civilization. Heat related deaths and mental illnesses are described in the ancient Indian literature which dates back to 3000 BC as well as during Biblical times (*Judith* 8: 2). One of the common causes of external heat injury is associated with the problems of fire, burning and rescue operation[1]. The other factors inducing heat illness are exertion in hot environment[2–4]; heat treatment of brain tumours[5–7] and/or prolonged high fever associated with bacterial or viral infections[8,9]. The deleterious effects of heat cause marked alteration in brain function and metabolism[10–13].

The term 'Heat Stress' usually denotes perceived discomfort and physiological strain associated with exposure to a hot environment (air temperature $>32^{\circ}C$) due to physical exercise or daily work for long periods[14,15]. However, a rise in core body temperature above the hypothalamic set point is known as 'Hyperthermia'. This is caused by an impairment of heat-dissipating mechanisms due to external (high environmental temperature) or internal (metabolic heat production, drugs or disease) factors[16,17]. The term 'Mild' to 'Moderate' hyperthermia is defined when

* To whom correspondence should be addressed. e-mail: sharma@medcellbiol.uu.se

International Journal of Hyperthermia ISSN 0265–6736 print/ISSN 1464–5157 online
http://www.tandf.co.uk/journals
DOI: 10.1080/0265673021000054621

the body temperature exceeds 37°C but usually remains below 40°C[16]. This mild-to-moderate heat illness, often known as 'Heat Exhaustion' may result from exposure to high environmental heat or strenuous physical exercise in a hot environment[14]. The signs and symptoms of heat exhaustion include intense thirst, weakness, discomfort, anxiety, dizziness, fainting and headache[16]. The body temperature may be normal, below normal or slightly elevated than 37°C[14,15]. The term 'Heat Stroke' is often used in the case of severe heat illness characterized by a rise of core body temperature >40°C associated with CNS abnormalities[3,4,14,15]. In such cases, delirium, convulsions or coma occur as a result of exposure to environmental heat (classical heat stroke) or strenuous exercise in hot environment (exertional heat stroke)[14,15].

Heat-stroke is one of the most severe illnesses caused by exposure to the high ambient temperatures[11,13,14,18,19]. Heat induced brain injury is the third largest killer of the young and adult athletes followed by cardiovascular and traumatic insults[14,18]. In spite of the seriousness of this problem, studies regarding effects of heat on the central nervous system (CNS) have been greatly overlooked and the mechanism(s) behind heat injury are still unclear.

Few sporadic reports describe post-mortem changes in the CNS of heat stroke victims[1,3,9,19]. These studies indicate that CNS is one of the most vulnerable organs following hyperthermia induced heat stroke[3]. On the basis of one's understanding of the pathophysiology of heat stroke, Bouchama and Knochel[14] suggested that 'heat stroke' can be considered as a form of hyperthermia associated with a systemic inflammatory response leading to a syndrome of multiorgan dysfunction in which encephalopathy predominates[14,15]. Although CNS pathology in hyperthermia associated with heat stroke has been described[1,3,9,15,19], the important components of the brain such as hippocampus and vascular endothelium were not examined. Moreover, ultrastructural investigations of the CNS in hyperthermia were not performed at all. Since, at that time, the functional significance of the blood–brain barrier (BBB) was not well characterized, the problems of microvascular permeability and oedema were largely ignored.

Whole body hyperthermia (WBH) is also used as a 'Therapeutic Hyperthermia' to destroy deep seated tumours in cancer patients[5–7]. This method induces a relative fast increase in the body temperature, in contrast to exposure to hot environments and heat stroke[14]. There are several side effects of therapeutic hyperthermia; however it is still effective as one of the potential therapies for cancer treatment[5,6].

In this review, effects of WBH induced changes in brain pathology in humans and animal studies are critically examined. In addition, new data on the influence of age, anaesthesia and repeated exposures to heat on brain dysfunction that is mainly based on our own investigations, are described.

2. Heat stress influences information processing system of the CNS

Heat stress (HS) appears to be a disorder of the thermal information processing system (IPS) that is chiefly regulated in the hypothalamus[16,17]. Excessive heat overload impairs the cooling mechanisms resulting in clinical hyperthermia which, depending on its magnitude and severity, leads to brain dysfunction. The probable link between the IPS, hyperthermia and abnormal brain function is still not well defined. Neurochemicals and cell signalling pathways appear to play key roles in the IPS and cell injury, probably through altered BBB function in hyperthermia[12,13,20]. Leakage of serum proteins across the BBB resulting in vasogenic

brain oedema formation is one of the most crucial events determining the extent of cell injury or cell death leading to brain pathology[12,13,20–23].

2.1. *Leaky BBB in heat related disorders*

It appears that stress induces brain dysfunction, probably through modification of the BBB. This is evident with the fact that a threefold increase in the frequency of neurological symptoms, e.g. headaches, insomnia, drowsiness, nervousness, unfocused attention and impaired capacity to conduct simple calculations occurred following pyridostigmine (a carbamate inhibitor of acetylcholinesterase which is used in military personal as antidote of nerve gas poisoning) ingestion by 213 Israeli soldiers as a treatment during the Persian Gulf War[24]. These soldiers serving in the Gulf War may have developed leaky barriers due to hot weather conditions prevailing in that region[25]. This idea is further supported with the fact that only a 50% dose of the peripherally administered pyridostigmine is needed to inhibit brain acetylcholinesterase in mice that were subjected to forced swim stress for 15 min compared to the control group[26]. These results confirmed the original observations of Sharma and his group that immobilization, forced swimming and heat stress causes breakdown of the BBB and is able to enhance protein extravasation in the brain in a highly specific and selective manner[27–34]. Taken together, these data support the hypothesis that the BBB permeability and drug transport in the nervous system are enhanced by mild hyperthermia and in other stressful situations.

3. Hyperthermia in humans

Documented heat induced CNS pathology following heat illness is limited[3,19,35]. Few sporadic case reports in the past describe brain histopathology following heat-stroke induced hyperthermia (6 h to several weeks) that are based on either Cresyl Violet or Haematoxyline and Eosin (H&E) staining of the paraffin embedded specimens[3,19]. No detailed histopathological, immunopathological information or ultrastructure changes in the brain are available.

3.1. *Pathological changes in the human brain*

The following description of brain pathology is based on various case reports in heat illnesses[3,4,19,36]. Post-mortem autolysis does not appear to play significant roles since similar changes were observed in cases ranging from 6–15 h after the death of heat-stroke victims (table 1).

3.2. *Cerebral cortex*

Oedema, congestion and degenerative changes in the neurons were quite prominent in the frontal cortex 11 h after heat injury (table 1). The nerve cells and their dendrites were swollen and the nucleus showed disintegration and chromatolysis. Some neurons were shrunken and the cytoplasm and nuclei were hyperchromatic. Glial cells appeared to proliferate in the cases of 24 h heat injury and onwards. At this time, severe neuronal loss was seen. In cases of 6 and 12 days of heat illness, the neuronal loss was most severe and the glial proliferation was quite distinct. These changes were most prominent in the upper layers of the cerebral cortex compared to the lower layers. Increased lipid content in neurons and in the perivascular space was quite common.

Table 1. Effect of heat on central nervous system damage in clinical cases. These pathological observations are based on light microscopy using Crersyl violet stain or Haematoxyline and Eosin staining. No immunohistochemistry was applied and ultrastructural investigations were not done.

Duration of heat illness	Brain regions	Cell damage		
		Nerve cells	Glial cells	Myelin
11 h	Cerebral cortex	oedema congestion disintegration	not known	not known
18 h		shrunken hyperchromatic		
276 h		degeneration	proliferation of microglia and astrocytes	
96 h		haemorrhages in white matter		
5 h	Cerebellum	Purkinje cells swollen, disintegrated	proliferation of oligodendrocytes	
5.5 h		oedema, swollen and loss of Purkinje cells		
72 h		Purkinje cells completely disappeared	glial proliferation	
276 h		loss of Purkinje cells granular cell layer	glial proliferation	
		degeneration of Purkinje cells	phagocytosis by glial cells	
11 h	Dentate nucleus	hyperchromatic, capillary engorgement		
276 h		few shrunken neurons	extensive gliosis	
276 h	Thalamus	neuronal loss	proliferation of glia	
4 h	Hypothalamus	microhaemorrhages only in PVN		
8 h		no significant change		
130 h		no significant change		
14 h		no significant change		
5 h	Midbrain	microhaemorrhages in the floor of IV ventricle, PAG, OM		

After Sharma *et al.*[20].

Compiled after Malamud *et al.* (1946)[3].

PAG = periaqeductal gray matter, PVN = paraventricular nucleus, OM = occulomotor nucleus, h = hours.

3.3. *Cerebellum*

Oedema of the Purkinje cell layer was most marked and the number of Purkinje cells was considerably reduced within 24 h of heat injury. Interestingly, the molecular and granular layers of the cerebellum were not affected except some minor proliferation of the satellite oligodendroglia. Twenty-four hours after heat illness, the Purkinje cell layer almost completely disappeared and glial reactions were pronounced in the Bergmann layer and the molecular layer. After 3 days of heat illness, the Purkinje cell necrosis was quite common. Hyperplastic changes were apparent in

molecular and Bergmann layers in cases of a 12 day heat illness, and the Purkinje cells were consumed by macrophages. These changes were found in both the cerebellar hemispheres as well as in vermis. The dentate nucleus exhibited signs of degeneration of nerve cells and hyperchromatic reactions 12 h after heat illness.

3.4. *Basal ganglia*

Changes in basal ganglia are less severe than in the cortex. The corpus striatum and thalamus were not much affected by heat injury. Only a few neurones in the caudate nucleus and putamen were diffusely damaged in short periods of heat illness. In the thalamus, focal collection of glia was seen and proliferation of microglia around the nerve cell was quite common in longer duration of heat illness. The globus pallidus was the least affected region of the brain in these cases and the periventricular system was devoid of glial proliferation.

3.5. *Hypothalamus*

Great attention has been paid to hypothalamus in heat injury, since this part of the brain is the main seat of thermoregulation[9,16,17,37]. A mild-to-moderate degree of oedema of the hypothalamic nuclei is seen during short duration of heat illness. Loss of neurons and a slight increase in the number in glial cells occur following in hypothalamus, irrespective of the duration of heat illness.

3.6. *Mid-brain, pons, medulla oblongata and spinal cord*

Mild changes in the nerve cells and a slight increase in the number of glial cells were observed in the quadrigeminal region, the inferior olivery nuclei and the reticular formation. No distinct cell changes or odema was seen in other parts of the hind brain (table 1).

3.7. *Microhaemorrhages*

Microhaemorrhages in leptomeninges and in other brain regions were mainly confined to the perivascular space in heat injury. The leptomeningeal haemorrhages were most diffuse and severe in short duration heat illness. Pronounced haemorrhage is seen in the paraventricular nucleus, the supraoptic nucleus, medial parts of the ventromedial and dorsomedial hypothalamic nuclei. Perifornical and septal regions and the medial portion of the thalamus as well as the caudal part of the hypothalamus were least affected. No haemorrhages were observed in the mammillary body. Haemorrhages of the pons and medulla oblongata were mainly confined to the floor of the fourth ventricle and sometimes near the dorsal efferent nucleus of the vagus.

3.8. *Brain oedema in clinical heat illness*

Oedema is the most prominent clinical feature following hyperthermic brain injury. A flattening of convolutions and a cerebellar pressure cone along with softening of the brain tissue are quite common[3,19]. In a few cases, brain weight was found to be increased by several hundred grams[3] following heat-stroke. Oedema of the leptomeninges was quite distinct.

4. Therapeutic hyperthermia

As mentioned above, 'Therapeutic Hyperthermia' has been employed for spontaneous cure to several kinds of tumours since the mid-19th century[38]. The potential

benefit of WBH is to destroy deep-seated tumours and the device may be effective alone against metastasis.

However, in such clinical trials, fluid loss, haemodynamic alterations, serum enzyme abnormalities and other symptoms of variable severity have been described[39]. Several fatalities occurred in this form of treatment, which is attributed to different causes rather than hyperthermia *per se*. Interestingly, the effect of WBH on the structure and function of the normal CNS has still not been worked out in details. Thus, the effective exposure temperature and CNS dysfunction over time are not known in most laboratory animals[40,41]. In addition, the effect of anaesthesia and age on WBH induced changes in brain function are still unclear. The time–temperature relationship with repeated systemic heat exposure and the development of thermal tolerance following repeated heat exposure is still not known[38]. The lacunae in this kind of experiments are that the brain histopathology following WBH is not investigated in detail.

4.1. *Effect of local hyperthermia on the CNS*

In several cases of localized hyperthermia applied on some parts of the brain or spinal cord, higher temperatures can be tolerated well by a small volume of nervous tissues[42,43]. Induced local hyperthermia used in cancer therapy is generally aimed to treat the brain tumour tissue with a heat dose in the range of 30–60 min at a temperature of 42–43°C. Such heat doses may lead to haemorrhages, necrosis to the surrounding normal nervous tissue with limited whole body physiological changes[44]. There are indications that local or regional heating of the brain can induce oedema formation and cell injury[42,43]. Pathological findings in these investigations are limited due to failure of obtaining brain tissue specimens. In some cases, haemorrhages, necrosis, inflammation and gliosis are described[42]. In these experiments, lethality after hyperthermic insults to the brain was very common[42]. However, the real cause of such phenomena is not known. There are reasons to believe that hyperthermia *per se* can induce such lethality, although this issue is still not well attended.

4.2. *Local heat treatment of CNS tumours in humans*

Little is known regarding the effect of local hyperthermia in the human nervous system. A tumour temperature of 42.5°C for 1 h was not found to be associated with any adverse effect. However, during this procedure, normal tissue temperature was not measured[37]. In a separate study by Tanaka *et al.*[45] in which the tumour tissue was heated up to 44–49°C, the normal tissue temperature did not exceed 38–40°C. In this study, the heat treatment resulted in aggravation of peritumoural oedema and a focal brain swelling. In contrast to this study, deep heating was applied in many patients without apparent heat neurotoxicity[46].

One possibility that heat toxicity did not develop in these studies is that most of the experiments was conducted under anaesthesia. Thus, the passive heating of brain may be an entirely different phenomenon rather than the stress associated with heating. It is important to note that most of the anaesthetics used in these investigations like pentobarbital and ketamine are known neuroprotective agents following various forms of ischemic or metabolic insults to the nervous system[47]. However, to get further clarification of the effects of anaesthetics on brain dysfunction following hyperthermia, additional work is required.

5. Effect of whole body hyperthermia on the CNS

5.1. *Clinical observations*

The effects of WBH on human brain function are largely unknown[43]. Few clinical investigations on brain tumour patients showed that if the brain temperature exceeds 39°C the neurological outcome is worsened. Mellergard and Nordström[48] showed that rectal temperature adequately reflects the epidural space temperature, which is very similar to that of tympanic membrane temperature[49].

WBH in humans induce a co-ordinated reaction, which involves endothelial cells, leukocytes and epithelial cells to protect against tissue injury and to promote repair[50]. A variety of cytokines are produced following endogenous or environmental heat[51]. Thus, WBH increases peripheral interleukin (IL)-1β, IL-6, IL-8, tumour necrosis factor-alpha (TNF-α) and the regulatory cytokine IL-10[14,52]. These cytokines modulate local and systemic inflammatory responses by controlling the levels of other cytokines[53].

Based on these observations, various studies suggested to treat human tumours using fever-range whole body hyperthermia (FR-WBH)[14,49–53]. In a recent study using FR-WBH by Kraybill *et al.*[54] showed that patients with advanced solid tumour tolerated well 39–39.5°C for 3 or 6 h and 39.5–40°C for 6 h without having any adverse effects on the cardiac, hepatic or renal systems. However, in these reports biopsy findings or study of other brain functions like cerebral blood flow (CBF) or other brain functions are not available.

5.2. *Brain pathology following WBH*

The brain pathologies occurring in heat stroke and the WBH are not comparable. However, hyperthermia induced either by heat stroke or WBH is quite similar in nature. Hyperthermia of 40.5–43°C for 3–11 h following WBH results in fatality and visible organ injury[55]. On the other hand, heat stroke induced fatalities can be seen when the rectal temperatures on admission ranged between 38–44°C. The death occurred in 70% of cases in less than 24 h (rectal temperature 41.5–44°C) in either WBH or heat stroke. On the other hand in benign cases, the survival period was between 1–12 days. It is interesting to note that similar changes in brain pathology were described by Malamud *et al.*[3] and Gauss and Meyer[1] in cerebellum regarding loss of Purkinje cells. This supports the idea that the brain pathology in hyperthermia either produced by WBH or heat stroke is quite similar in nature. Lack of immunohistochemistry or ultrastructural assessment in these cases makes the interpretation difficult. Furthermore, other important organs of the brain such as hippocampus, endothelial cells and spinal cord were not examined. Thus, this subject requires further investigation.

6. Effect of hyperthermia on brain energy metabolism

The influence of brain energy metabolism in hyperthermia is the obvious concern to the clinicians who encounter hyperthermia as a common complication as well as aggravating a variety of cerebral disorders including traumatic injuries to the CNS. In humans, if hyperthermia exceeds 40°C, symptoms of nausea, disorientation, apathy and delirium appear. If the hyperthermia is severe enough, it may result in heat stroke and the body temperature may reach >42°C[10]. At $\sim$42°C, the signs of cardiovascular dysfunction associated with a fall in the blood pressure occurs[56]. A small increase in the temperature further directly damages neurones, probably because of heat inactivation of enzyme proteins, even in the absence of overt

circulatory failure[10] followed by convulsion, coma and death[19,55,57]. These symptoms are caused by excessive accumulation of body heat[58].

6.1. *Pulmonary ventilation and arterial $PaCO_2$ changes*

When the body temperature exceeded 41°C, the accumulation of lactate in blood occurs indicating acid production by anaerobic glycolysis. The development of respiratory alkalosis may help in preventing further increase in the CBF due to hyperthermia that may have direct pathophysiological consequences in the brain.

Hyperventilation, an increase in blood pH (alkalosis) as well as a decrease in arterial $PaCO_2$ occurs in hyperthermia[56,59,60] (table 2). The rate of fall in $PaCO_2$ is steep in conscious animals compared to the animals exposed to heat stress under anaesthesia[10].

6.2. *Metabolic rates and blood flow changes*

A progressive increase in temperature from 40–43°C induces a decline in oxygen uptake with time[61]. If the temperature is >45°C and prolonged, then the depression of metabolic rate is irreversible[61] leading to cell injury[10,62]. An increase in rectal temperature by 2°C in humans during asymptomatic neurosyphlis did not influence the cerebral metabolic rate for oxygen consumption ($CMRO_2$)[63] (table 2). However, in patients with dementia paralytica, a reduction in $CMRO_2$ is evident at normal temperature. In dogs, an increase in body temperature from 37.8–41.5°C is associated with a 50% rise in $CMRO_2$ (from 2.2–3.3 mol/g/min)[64].

The CBF remained unchanged in hyperthermia in spite of marked reductions in $PaCO_2$. This indicates that the vasoconstrictory effects are outbalanced by vasodilatation due to increased temperature[60,65,66]. In rats, CBF and $CMRO_2$ shows an increase in the order of 5% per degree rise in the body temperature up to 42°C, if the $PaCO_2$ is kept constant[10,67].

Table 2. Effect of hyperthermia on cerebral energy metabolism and physiological variables.

Species	Anaesthesia	Rectal T°	Variables measured		
Pulmonary ventilation and arterial $PaCO_2$					
Dog	Barbiturate	>41°C	20 mm Hg↓	Respiration↑	pH↑
Oxen	—	>41°C	<20 mm Hg↓	Respiration↑	pH↑
Sheep	—	>41°C	<10 mmHg↓	Respiration↑	pH↑
Metabolic rates and blood flow					
Humans	—	>Δ2°C	$CMRO_2 \neq$	Neurosyphlis	
Humans	—	normothermia	$CMRO_2 \downarrow$	dementia paralytica	
Humans	—	>Δ2°C	$CMRO_2 \uparrow$	dementia paralytica	
Dogs	Pentobarbital	41.5°C	$CMRO_2 \uparrow$	CBF≠	
Dogs	Pentobarbital	>41°C	CMRgl↑;↓;≠		
Rat	Barbiturate	40°C	$CMRO_2 \uparrow$	CBF	
Rat	Barbiturate	42°C	$CMRO_2 \uparrow$	CBF≠;↑	
Sheep	—	42°C	$CMRO_2 \downarrow;\uparrow$	CBF≠	
Intermediary metabolism					
Rats	Barbiturates	42°C	ADP↑	Lact/Pyr↑	
Rats	Barbiturates	42°C	Glu-6-P↑	Fru-6-P↑	

≠ = no change; ↑ = increase; ↓ = decrease.

$CMRO_2$ = Cerebral metabolic rate for oxygen consumption; CMRgl = Cerebral metabolic rate of glucose consumption; CBF = Cerebral blood flow.

Compiled from Various Sources[10,12,67].

6.3. *Changes in intermediary metabolism*

In unanaesthetized mice, an increase in body temperature to 43–44°C is associated with reductions in PCr and ATP and increases in ADP and AMP[10,67]. However, in the rat, no changes in PCr, ATP or AMP can be seen and only a small rise in ADP and the lactate/pyruvate ratio was observed (table 2)[67]. It seems likely that 42°C represents a critical temperature above which energy failure in brain develops leading to cell autolysis[10].

7. Whole body hyperthermia and brain dysfunction

Several workers did not observe heat toxicity in the brain after WBH, although the treatment caused lethality[68,69]. Based on these observations, it has been suggested that CNS is not an organ at risk after WBH. One probable reason could be that the rise in brain temperature is not uniform after whole body heating. Electrophysiological studies showed profound alteration in brain electrical activity after WBH[69,70], indicating that CNS is a very sensitive organ in hyperthermia. Since, in these studies, histopathological examination of the CNS is not done, the subjects still remain controversial.

On the other hand, studies carried out in the laboratory during the last two decades show that the CNS is highly vulnerable following WBH in conscious animals[27–34,71–84]. The observation further suggests that damage of CNS depends on the magnitude and severity of heat exposure, the amount of thermal load and the physiological states of animals prior to heat exposure[74,77].

7.1. *Chronic summer heat exposure*

In order to simulate chronic summer heat conditions, animals were exposed to the ambient air temperature of Varanasi (relative humidity 50–55%, wind velocity 24.5 cm/s) from 8.00 am to 6.00 pm for 1 week[28–30] and the BBB permeability was examined using Evans blue albumin as exogenous protein tracer.

Exposure of young rats (age 8–9 weeks old) to chronic summer heat at 31–33°C caused a mild thermal load ($+0.84 \pm 0.23$°C) without showing any increase in the permeability of the BBB. On the other hand, when young rats were exposed chronically to summer heat at 34–36°C, the permeability of the BBB was increased in more than 60% of animals. These rats experienced a greater degree of thermal stress ($+2.5 \pm 0.46$°C). Old rats (age 24–32 weeks) subjected to similar heat exposure did not show either any increase in the BBB permeability or thermal stress ($+1.8 \pm 0.23$°C)[27,28]. These results were the first to show that chronic summer heat can induce breakdown of the BBB that is most pronounced in young age groups[26]. The results opened a new vista of brain research, indicating that the metabolism and pharmacokinetics of drug transport to the brain are altered in summer heat conditions, a finding which is confirmed by several independent workers recently[24–26].

7.2. *Acute heat exposure*

Since summer heat resulted in a leaky BBB, the effect of thermal stress was further examined in a controlled manner by exposing rats in a biological oxygen demand (BOD) incubator[28–30,32,71,85].

7.2.1. *The rat model.* Brain dysfunction in several animal models are characterized by exposing rats to high temperatures ranging from 32–46°C for a period of 30 min to 8 h in acute experiments and several days at 30–34°C for chronic

Table 3. Stress symptoms and physiological variables in control and heat stressed rats.

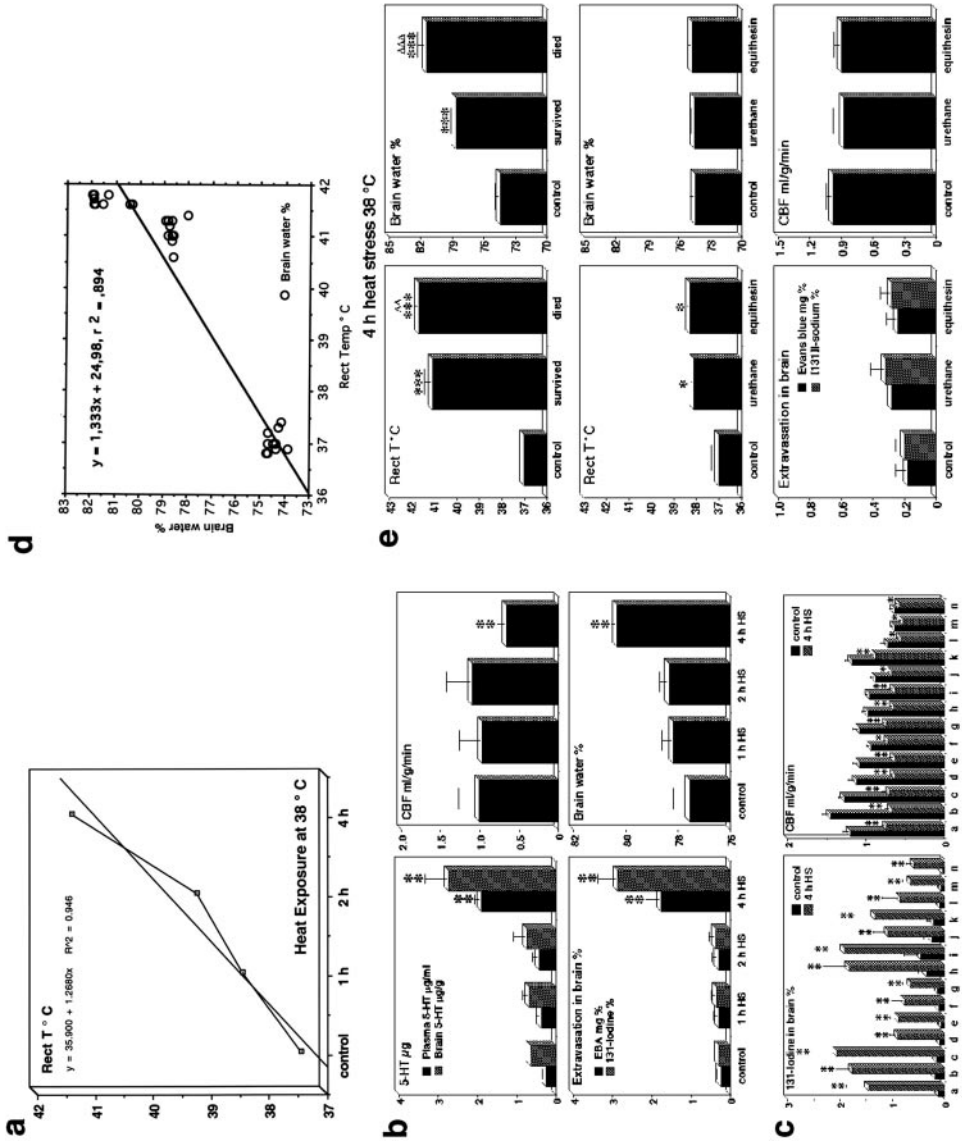
Parameters examined	Control[a] ($n = 8$)	Heat stress at 38°C in BOD chamber		
		1 h ($n = 6$)	2 h ($n = 8$)	4 h ($n = 12$)
Stress symptoms				
Rectal T °C	37.42 ± 0.23	38.41 ± 0.32*	39.24 ± 0.21**	41.48 ± 0.23***
Salivation	nil	++	+++	++++
Prostration	nil	nil	nil	++++
Gastric haemorrhage	nil	4 ± 3	8 ± 3	34 ± 8 (microhaemorrhages)
Physiological variables				
MABP torr	101 ± 6	94 ± 8	124 ± 8**	76 ± 4**
Arterial pH	7.38 ± 0.04	7.36 ± 0.03	7.33 ± 0.10	7.34 ± 0.08
$PaCO_2$ torr	33.46 ± 1.04	33.56 ± 0.76	34.13 ± 0.24	32.12 ± 0.11*
PaO_2 torr	78.24 ± 1.22	79.12 ± 0.54	79.34 ± 0.26	82.14 ± 0.23**

[a]Control rats kept at room temperature (21 ± 1°C); Rats exposed in a biological oxygen demand (BOD) incubator at 38°C for heat stress (for details see text); nil = absent, ++ = mild, +++ = moderate, ++++ = severe; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ Student's unpaired t-test (Data modified from Sharma[28], Sharma and Dey[30–32], Sharma *et al.*[20]).

studies[69,86–92]. In some animal models, extremely high temperature (usually 41–44°C) was used to induce neurological symptoms of acute heat stroke. In these models, the death rate is very high (more than 80%) and study of brain dysfunction *in situ* is not possible[20,93].

A new rat model was developed in which animals are exposed to heat at 38°C in a biological oxygen demand (BOD) incubator for 4 h. The relative humidity (45–50%) and wind velocity (20–25 cm/s) is kept constant[28]. This experimental set up resulted in stress symptoms and physiological variables (see table 3) very close to that of clinical situations. Exposure of rats to either 30 or 34°C in the BOD incubator resulted in only mild hyperthermia (figure 1(*a*)), indicating that the model is based

Figure 1. Heat stress induced modification of body temperature, BBB permeability, serotonin levels, CBF changes and brain oedema formation in conscious and anaesthetised rats (*a*). Rectal temperature in control, 1, 2 and 4 h heat stressed rats exposed at 38°C. Each point represents mean ± SD of 6–12 rats. (*b*) Blood–brain barrier permeability, brain oedema (lower panel), cerebral blood flow and 5-HT level (upper panel) in control and heat stressed rats (** = $p < 0.01$ Student's unpaired t-test, compared to the control group) (*c*) Regional blood–brain barrier permeability and regional cerebral blood flow in control and 4 h heat stressed rats. Values are mean ± SD of 12–14 rats. a = frontal cortex, b = parietal cortex, c = occipital cortex, d = cingulate cortex, e = hippocampus, f = caudate nucleus, g = thalamus, h = hypothalamus, i = superior colliculus, j = inferior colliculus, k = cerebellum, l = pons, m = medulla. * = $p < 0.05$, ** = $p < 0.01$, compared to controls (ANOVA followed by Dunnett test) (*d*) Shows correlation between increased body temperature (%, x-axis) and increased brain water content (%, y-axis) in control and 4 h heat stressed rats. The regression line showed strong correlation between hyperthermia and brain oedema development ($r^2 = 0.968$, $p < 0.001$), (*e*) Effect of 4 h heat stress induced brain function in conscious (upper panel), and urethane or equithesin anaesthetised rats (middle panel) and BBB permeability and CBF (lower panel). Values represent mean SD of 5–6 rats. * = $p < 0.05$, *** = $p < 0.001$ compared from control group, ΔDD = $p < 0.001$ compared from survived group (upper panel) (data modified after Sharma and Dey[32], Sharma *et al.*[20,71], Sharma[12]).


a
Rect T° C
y = 35.900 + 1.2680x RV2 = 0.946
Heat Exposure at 38 ° C
control
1 h
2 h
4 h
b
5-HT µg
Plasma 5-HT µg/ml
Brain 5-HT µg/g
CBF ml/g/min
Extravasation in brain %
EBA mg %
131-Iodine %
Brain water %
control
1 h HS
2 h HS
4 h HS
c
131-Iodine in brain %
CBF ml/g/min
control
4 h HS
d
Brain water %
y = 1,333x + 24,98, r 2 = ,894
Rect Temp ° C
e
4 h heat stress 38 °C
Rect T°C
Brain water %
control
survived
died
urethane
equithesin
Extravasation in brain
Evans blue mg %
131I-sodium %
CBF ml/g/min

on physiological aspects of noxious heat stimulus not associated with skin injury and has markedly less mortality (less than 20%, if any).

7.2.1.1. *Behavioural symptoms and lethality*. At the end of 4 h heat exposure at 38°C some rats lay prostrate in cages and did not move even after gentle pushing[29,30]. However, these rats did not lose their righting reflex[26]. Some rats whose body temperature exceeded 42°C died during or a few min after termination of heat exposure[28,71]. Some animals, which survived 5–10 min after heat exposure, exhibited low mean arterial blood pressure (MABP, 60–65 torr) close or below the critical *lower limit* of the cerebral autoregulation, indicating that these rats developed brain ischemia due to defective autoregulation of the CBF[94]. Post-mortem studies showed fluidity of all the cerebral components[71]. Damage of vital centres due to volume swelling of the brain in a closed cranial compartment appears to be the main cause of death[71]. Obviously, this model can be used to study brain pathology in heat stress and to explore new strategies of various therapeutic measures to achieve neuroprotection[20,95,96].When rats were exposed to 39°C in a BOD incubator, most of the animals died within a 2 h session (Sharma, unpublished observation). However, the details of the maximum tolerated dose after WBH in rats, and how indicative is this for human situations is still not known.

7.2.1.2. *Stress symptoms and physiological variables*. Exposure of young rats in a BOD incubator at 34 or 36°C for 4 h resulted in a small rise in their rectal temperature ($+1.8 \pm 0.12$°C and $+2.4 \pm 0.18$°C, respectively) and exhibited only minor salivation. Few animals exhibited microhaemorrhages in their stomach wall[28]. Rats subjected to 4 h heat stress at 38°C in the BOD chamber resulted in profound hyperthermia ($+3.64 \pm 0.57$°C) and stress symptoms. Post-mortem examination showed many haemorrhagic petachae in the stomach wall. In these animals, MABP fell by 20 torr from the basal value. The PaO_2 showed a mild increase, whereas the $PaCO_2$ and arterial pH were slightly decreased (table 3).

On the other hand, rats exposed to 38°C for 1 or 2 h showed a mild degree of stress symptoms[30,32], indicating that the magnitude and severity of stress symptoms are dependent on the amount of thermal load and duration of heat exposure (table 3).

7.2.1.3. *Blood–brain barrier permeability changes*. Exposure of rats at 34 or 36°C in a BOD incubator did not result in the breakdown of the BBB permeability[28,32] to Evans blue and radioactive iodine[131] I-sodium tracers[28,29]. These tracers bind to serum albumin into the circulation, and a leakage of these tracers across the BBB thus represents extravasation of tracer-protein complex[28,30,32,85,94].

On the other hand, rats exposed to 4 h heat stress at 38°C showed a marked increase in the BBB permeability to Evans blue (figures 1(*b*) and (*c*)) and radioactive iodine (figure 1(*d*)). This increase in the BBB permeability was absent in animals subjected to 1 or 2 h periods of heat exposure. The observations demonstrate that heat stress has the capacity to induce a widespread increase in the permeability of the BBB to protein tracers depending on its magnitude and duration.

7.2.1.4. *Regional BBB permeability*. In order to find out a selective vulnerability of brain regions sensitive to heat, the regional increase in the permeability of the BBB was examined in 14 areas of rat brain (figure 1). The Evans blue staining was noted in eight brain regions in order of decreased frequency ($n = 24$): cingulate cortex (99%), occipital cortex (96%), parietal cortex (94%), cerebellum (90%), temporal

cortex (88%), frontal cortex (85%), hypothalamus (78%) and thalamus (64%). The other brain regions were devoid of blue staining.

A significant increase in radioactivity was noted in all the 14 brain regions examined (figure 1(*c*)). Thus, besides the eight blue-stained regions, another six brain regions viz hippocampus, caudate nucleus, superior colliculus, inferior colliculus, pons and medulla also showed an increase in[131] I-sodium extravasation (figure 1(*c*)). This extensive increase in the permeability of the radiotracer compared to Evans blue dye appears to be due to differences in protein binding capacity of the former tracer or due to differences in proteins to which these tracers adhere in the circulation[30–32].

7.2.1.5. *Cerebral blood flow changes*. In order to gain new insight regarding contribution of cerebral circulation in BBB dysfunction, changes in global and regional CBF in heat stress were examined using tracer microspheres[32]. The measurement of CBF showed that subjection of rats to 4 h heat stress significantly reduced the global CBF by 30%, whereas heat exposure of 1 or 2 h did not influence the CBF changes compared to the control value (figure 1(*c*)). These observations suggest that CBF is influenced by heat stress, which in turn depends on the duration of heat exposure.

7.2.1.6. *Relationship between changes in regional CBF and BBB permeability*. Although the decline in the CBF was seen in almost all the regions which exhibited increased BBB permeability, the regional changes in the CBF and the BBB were unrelated. In five cortical and six sub-cortical brain regions, the regional CBF was significantly reduced by 38–53% and 23–31%, whereas the BBB permeability in these regions was increased by 87–1366% and 318–590%[11]. On the other hand, in cerebellum and brain stem, though the regional CBF declined only by 15–22%, the regional BBB permeability was increased by 844–1350%[12]. Thus, the intensity of flow reduction was not correlated with the magnitude of increased BBB permeability. A reduction in CBF, however, may influence brain metabolism and can contribute to the local cerebral energy changes[10] in heat stress which require further investigation.

7.2.1.7. *Brain oedema formation*. An increased permeability of the BBB resulting in extravasation of protein tracers in many brain regions is associated with vasogenic brain oedema formation[21,22,33,72,94]. Oedema is defined as an increase in the water content of the brain or spinal cord following noxious insults to the brain, mainly due to leakage of proteins across the microvessels[20]. In due course of time, the oedema fluid will spread and, depending on the magnitude and severity of the primary insult, whole brain can be swollen within 24 h[12,13,21]. A swollen brain in a close cranium will result in compression of vital centres leading to instant death[21].

Brain oedema was evaluated from changes in the brain water content and volume swelling calculation using the formula of Elliott and Jasper[97]. In general, an increase of 1% water content will reflect ~3% increase in volume swelling[21,33,94].

The results support the idea that brain oedema formation is closely associated with breakdown of the BBB permeability in heat stress. Rats did not show either oedema development or leakage of the BBB permeability at the end of a 1 or 2 h period of heat exposure (figure 1). However, 4 h heat exposure significantly resulted in the breakdown of the BBB permeability and leads to profound brain oedema formation (figure 1(*e*)). In these rats, the brain water content was increased by

4%, corresponding to ~16% increase in volume swelling[71]. The results are the first to show that acute heat exposure can induce brain oedema formation[71] and the magnitude and severity of brain swelling in the closed cranial compartment is responsible for heat-induced deaths[71] (figure 1). It remains to be seen whether the heat stress induced changes in the BBB permeability and brain oedema formation is reversible in nature.

7.2.2. *Serotonin as a neurochemical mediator of hyperthermic brain injury*. Serotonin was measured as one of the most potent neurochemical mediators involved in breakdown of the BBB permeability and brain oedema formation[21,23,85,98–100] using a sensitive and specific spectrophotofluorometric method[30,101]. The serotonin level was increased in the plasma (+656%) and in brain (+328%) after 4 h heat stress[11,30,59] (figure 1(*c*)). However, 1 or 2 h periods of heat exposure did not result in any significant increase in the serotonin level either in plasma or brain (figure 1). Regional brain serotonin level was diminished in the cortex, mid-brain and hippocampus, except cerebellum following acute exposure (1–2 h). Four hours after exposure, the serotonin level significantly increased in all the regions except the mid-brain. The plasma concentration of serotonin closely follows the changes in brain serotonin level at the end of 4 h heat exposure[85,102]. This indicates that the amine is positively involved in the hyperthermia induced BBB dysfunction.

However, in the *in vivo* situation, no single chemical compound appears to be responsible for all the changes seen in either experimental or clinical hyperthermia[20,103]. In fact, several factors or neurochemicals are simultaneously playing roles either to induce or counteract the effects of hyperthermia on BBB permeability and brain damage. Thus, further studies regarding involvement of other putative neurochemical mediators are needed in this model.

7.2.3. *Brain pathology*. Using immunohistochemical techniques at light and electron microscopy, morphological changes in the brain of heat stressed animals have been examined.

7.2.3.1. *Light microscopy*. Marked neuronal, glial, axonal and vascular reactions were present in several brain regions after heat exposure of rats at 38°C for 4 h[33,34,72,73,79,80,82,84]. Profound neuronal reaction using Nissl staining can be seen in many parts of the brain (figure 2(A)). Dark and distorted neurons were present in the cerebral cortex, brain stem, cerebellum, thalamus and hypothalamus. These changes were mainly present within the oedematous area of the brain. In several brain regions, neuronal cell loss was very common[34]. In the hippocampus, most severe changes in the nerve cells were noted in the CA-4 sub-field compared to other regions. Although oedematous swelling and general sponginess were present in the whole hippocampus, loss of neurons and degenerated nerve cells were most frequent in the CA-4 sub-field (figure 2(A.*b*)). This indicates that the phylogenetically oldest part of the hippocampus is particularly vulnerable to heat stress[20,80,82,103].

7.2.3.2. *Ultrastructural changes*. Ultrastructural studies of nerve cells in the cerebral cortex, hippocampus, cerebellum, thalamus, hypothalamus and brain stem show degenerated nucleus that is often accompanied by the eccentric nucleolus (figure 2(B.*b*)). The nuclear membranes exhibit many irregular foldings and the nucleolus often showed signs of degeneration. Several dark and dense structures are present in the nuclear or nerve cell cytoplasm[20]. In many parts of the cerebral cortex, hippo-

campus, cerebellum and brain stem, often one nerve cell is quite dark in appearance with condensed cytoplasm whereas the adjacent nerve cell is normal[20,73,104]. These observations indicate a selective vulnerability of nerve cells in heat exposure.

Several swollen synapses with damage to both pre- and post-synaptic membranes are present in the thalamus, brain stem, hypothalamus, cerebellum, cerebral cortex and in the hippocampus[80]. Oedema, membrane disruption, vacuolation, degeneration and distortion of the synaptic vesicles as well as damage of post-synaptic dendrites were most common findings (figure 2(B.*b*)).

Degeneration and vesiculation of myelin sheaths are frequent in several brain regions following heat stress[73,77,78]. Several unmyelinated axons were also swollen. Myelin damage together with swollen axons was present in the brain stem reticular formation, pons, medulla and the spinal cord[20,102].

7.2.3.3. *Vascular reaction*. Vascular reactions comprise partial to complete collapse of microvessels in several brain regions. Perivascular oedema, ischemic membrane damage and disruption of the BBB permeability to the electron dense tracer, lanthanum is quite frequent[20,33,79,82,103].

A highly selective nature of the endothelial cell membrane permeability was seen in heat stress[20]. Thus, several brain regions exhibit leakage of lanthanum across the cerebral endothelium (figure 2(C)). Moreover, in another brain region, leakage of the electron dense tracer is not evident. A particular vessel or often one endothelial cell shows leakage of lanthanum in one area, whereas the rest of the vessel or the adjacent endothelial cell does not show any evidence of the lanthanum exudation.

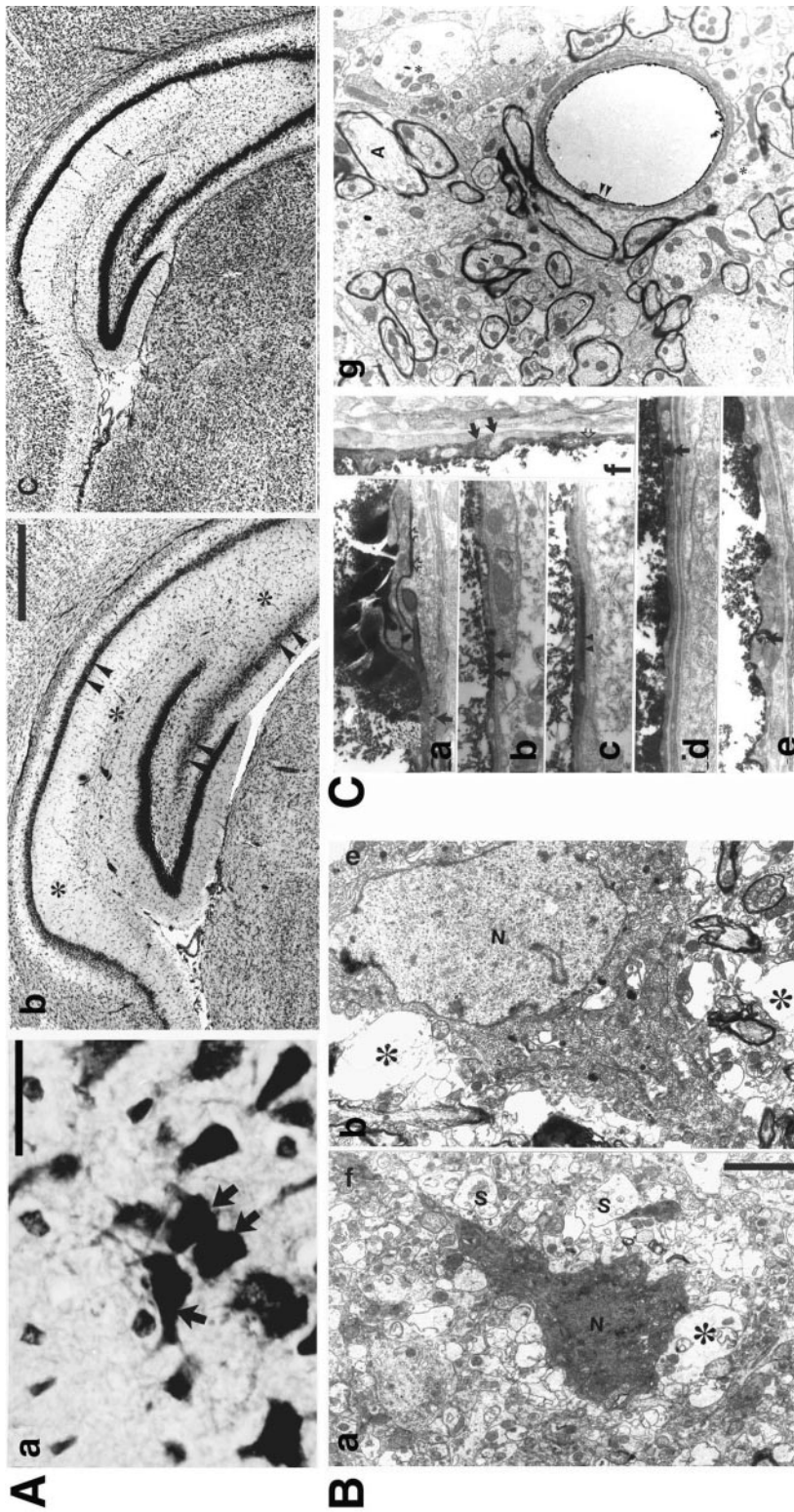
In large numbers of vascular profiles, lanthanum is stopped at the tight junctions, whereas the endothelial cell membrane is infiltrated with lanthanum containing tight junctions[20,103]. These findings support the idea of a specific receptor mediated increase in the endothelial cell membrane. Obviously, receptors are present on the membranes even on the apposing tight junctions[105,106].

7.2.3.4. *Ultrastructural aspects of the BBB permeability: tight junctions*. Many microvessels showed infiltration of lanthanum across the endothelial cells that are connected with tight junctions. In some cases, the membrane permeability across the tight junctions was increased without apparent deformation of these junctions (figure 2(C)). This evidence of increased permeability of the tight junctions to lanthanum in heat stress without widening them is entirely a new phenomenon not known before (for details see Sharma *et al.*[12,20]).

Tight junctions are formed between two apposing endothelial cell membrane layers[94,99,107,108]. An increase in the endothelial cell membrane permeability (as above) in these regions containing tight junctions can occur without apparently widening them.

7.2.4. *Immunohistochemical studies*. The molecular mechanisms of cell injury in the CNS of heat stress was examined using several immunomarkers recently identified as the key factors involved in brain pathology. These findings are summarized below.

7.2.4.1. *Axonal damage*. Myelin basic protein (MBP) was used to identify axonal damage[109]. A significant reduction in MBP indicating degradation of myelin in heat stress was observed in several brain regions[64]. This decrease in MBP immunostaining was prominent in brain stem reticular formation, pons, medulla and the spinal cord[20]


A
a
b
c
B
a
b
e
f
N
S
C
a
b
c
d
e
f
g

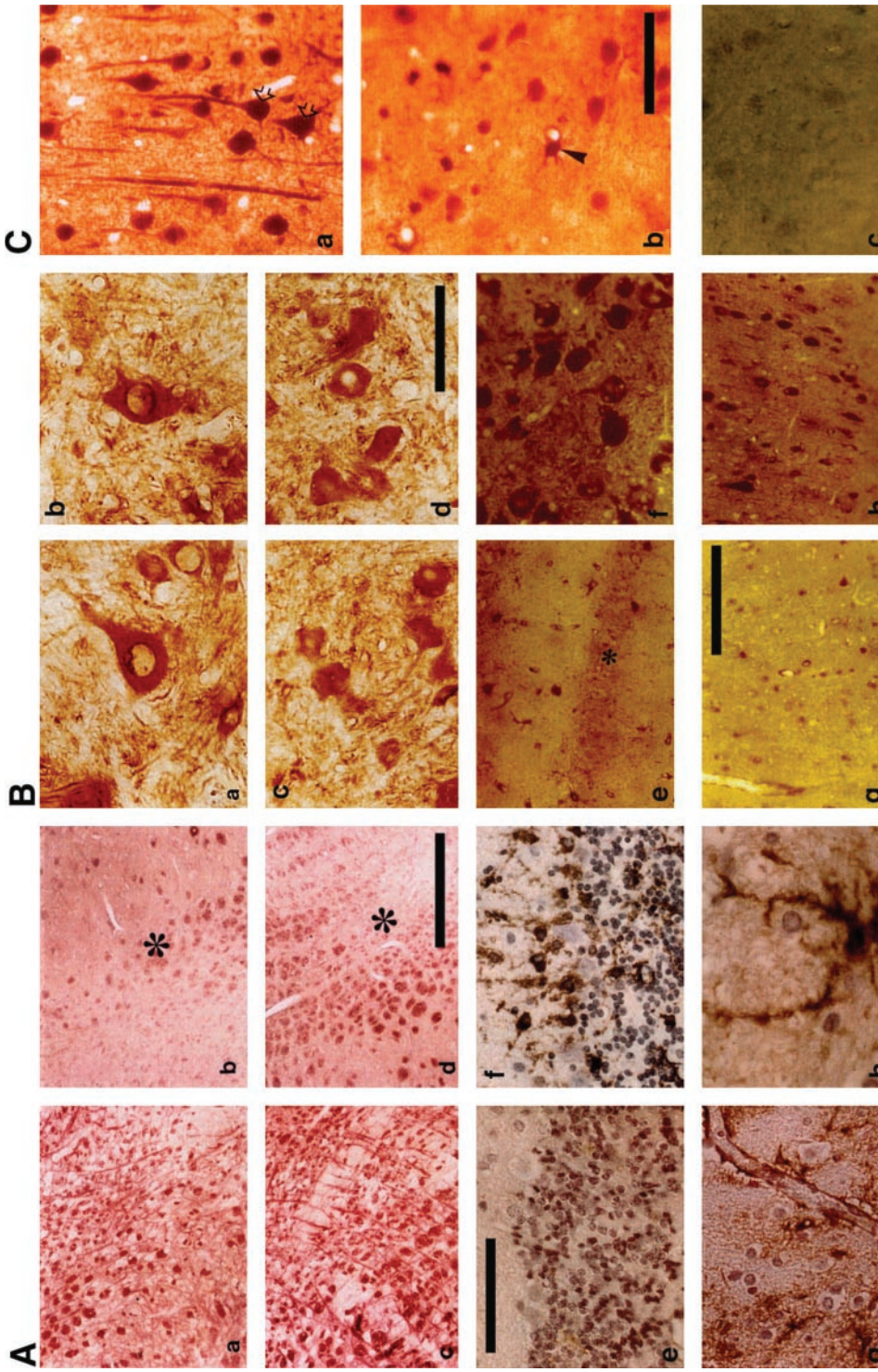
(figure 3(A.*a–d*)). Pre-treatment with drugs which induced neuroprotection are able to attenuate degradation of MBP immunoreactivity and myelin damage at the ultrastructural level[20].

7.2.4.2. *Glial cell reaction*. Immunostaining of glial fibrillary acidic protein (GFAP), a specific marker of astrocytes, was used to examine glial cell reactions in heat stress[110]. The GFAP-immunoreactivity was remarkably increased in several brain regions following 4 h heat stress and was often located around the blood vessels.

In the cerebral cortex, an increase in GFAP immunoreactivity occurs mostly in cortical layers III and IV[75,76]. In the hippocampus, the CA1, CA2 sectors and granular cell layer as well as in the lateral cerebellar nuclei and in the molecular layer of the cerebellum, strong GFAP positive astrocytes were present in (figure 3(A.*e–f*)). The Bergmann glia showed significant increase in immunoreactivity and GFAP positive astrocytes in granular layer are quite distinct[110]. In the brain stem, reactive gliosis was evident in the fasciculus solitarius, radial nucleus and tractus tectospinalis (figure 3(A.*g–h*)). The Raphé pontis and cross-sectioned pontine fibre-tracts showed strong GFAP-positive astrocytes. In the spinal cord, the intensity of GFAP immunostaining was mainly concentrated in the dorsal horn, ventral horn and around the central canal regions. GFAP-stained cells are often found around the blood vessels indicating an importance of vascular glial interaction in the pathological mechanisms of heat stress[20,75,76,82,95,110].

7.2.4.3. *Vimentin expression*. Normal adult rats did not show any signs of vimentin immunoreactivity in any brain region[20,76,78,95]. However, marked increase in the immunoreactivity for vimentin was seen in the brain stem reticulum formation, thalamus, cerebellum, corpus callosum and in some regions of the hippocampus. These observations suggest that heat stress induces selective upregulation of vimentin expression in adult rats as well[20,110]. An increased expression of vimentin in adult

Figure 2. A. Structural changes in the cerebral cortex, hippocampus (A), brain stem (B) and cerebral endothelial cells (C) following 4 h heat exposure at 38°C in young rats. (*a*) Nissl stained nerve cells in the cerebral cortex shows damaged and dark stained nerve cells (arrows) (bar = 30 μm). (*b*) Celloidin section (5 μm thick) of hippocampus shows prominent nerve cell loss in the CA 4 region (*b*) compared to the control (*c*) (bar = 200 μm). B. Ultratstructural changes in nerve cells (*n*) from brain stem (*a*) and parietal cerebral cortex (*b*) of heat stressed rat. Degeneration of nerve cells, cytoplasmic vacuolation (*a*) and increased density of cytoplasm and karyoplasm (*b*) are clearly visible. The nucleolus is completely degenerated. Signs of vacuolation, oedema (*) and synaptic damage (S) is quite frequent (bar a = 500 nm, b = 1 m) C. Passage of lanthanum across the cerebral endothelial cell membrane containing tight junctions in 4 h heat stressed rats. (*a*) Lanthanum is seen diffusely infiltrated within the cell membranes of tight junction complex and endothelial cell cytoplasm. However, lanthanum within the intercellular cleft is stopped at the tight junction (blank arrows, *a*); (*b*)–(*c*) A portion of the endothelial cell membrane is completely infiltrated with lanthanum (*b*: filled arrows, *c*: arrow heads) or the tracer is present within the vesicles of endothelial cell cytoplasm (*d*, *e*: filled arrow). In most cases one endothelial cell tight-junction shows diffusely infiltrated lanthanum (filled and blank arrows) leaving adjacent cell completely intact (*f*: filled arrows) (bar *a*, *e*, *f* = 200 nm, *b*, *c*, *d* = 300 nm). (C) *g* = One microvessel from the brain stem of a chronically heat stressed rats showing signs of heat adaptation. In heat adapted rats, cell damage, lanthanum extravasation, perivascular oedema or vesiculation of myelin are absent (bar = 1200 nm), from Sharma *et al.*[20], Sharma[12].


A
B
C
a
b
c
d
e
f
g
h

rats in heat stress suggests that the glial cells are activated following thermal brain injury and reflect the pathological alteration of astrocytes.

7.2.4.4. *Ultrastructural changes of glial cells*. Swollen astrocytic processes filled with disrupted bundles of glial filaments and glycogen particles are seen by electron microscopy in the heat injured oedematous brain tissues. Oedema allows the tight bundles of glial filaments to dissociate, resulting in more antigenic sites available to GFAP antibodies[12,110]. These observations support the idea that the glial cells are one of the important potential targets of heat induced brain damage.

7.2.4.5. *Nitric oxide and carbon monoxide*. The recently identified gaseous molecules nitric oxide (NO) and carbon monoxide (CO) are involved in the molecular mechanisms of cell injury[12,94,111,112]. However, their involvement in hyperthermic brain injury is not well characterized. The NO and CO are synthesized by the enzymes nitric oxide synthase (NOS) and heme oxygenase (HO), respectively, and are normally present within the normal CNS[12,83,94,111,112]. There is evidence that hyperthermia is associated with marked alterations in these enzymes (for review see Sharma *et al*.[95]). To study the involvement of NO and CO in the pathophysiology of hyperthermic brain injury, the expression of constitutive isoforms of NOS (nNOS) and HO (HO-2) was examined in the CNS using immunohistochemical methods in the rat model[83,113,114].

7.2.4.5.1. *nNOS and HO-2 immunoreactivity*. In normal rats, only a few neurons showed NOS immunostaining in the cerebral cortex, hippocampus, thalamus, hypothalamus and brain stem[83]. The spinal cord in general did not show NOS positive neurons[83,115]. On the other hand, a few HO-2 immunolabelled nerve cells are present in the spinal cord, cerebellum, cerebral cortex, thalamus and hypothalamus of the normal rats[12,83,95,113]. The number of HO-2 positive cells are considerably less compared to NOS positive cells in the control group.

Subjection of animals to a 4 h heat stress resulted in profound upregulation of nNOS and HO-2 positive neurons in many brain regions[12,83,95,113]. Upregulation of NOS immunoreactivity was found in many parts of the cortex, hippocampus, cerebellum, thalamus, hypothalamus and spinal cord, which do not normally exhibit nNOS activity. The immunostaining of NOS is clearly visible in neuronal cytoplasm.

Figure 3. A. Immunopathological changes in axons, astrocytes and nerve cells in rats following 4 h heat stress. (*a*) Degradation of myelin basic protein (MBP) in the brain stem reticular formation seen (*) as red staining is evident in stressed rat (*b*, *d*) compared to controls (*a*, *c*) (bar = 100 μm). (*e–h*) Upregulation of GFAP in cerebellum in following heat stress (*f*) compared to control (*e*). In brain stem reticular formation, strong GFAP signal is evident around an arteriole (*g*) and in the neuropil (*h*) following heat stress (bars: *e*, *f*: 50 μm; *g*, *h*: 25 μm). B. Upregulation of constitutive isoform of heme oxygenase-2 (HO-2) immunostaining in 4 h heat stressed rats (*a–d*). The HO-2 immunoreactivity is present in the cell cytoplasm and in the cell nucleus in the cortex (a), thalamus (b), brain stem (*c*) and spinal cord (*d*) (bar *a*, *b* = 10 μm, *c*, *d* = 20 μm). Upregulation of NOS immunoreactivity is also seen in the cell cytoplasm, and in the cell nucleus (f, h) following 4 h heat stress compared to controls (*e*, *g*). C. Upregulation of HSP 72 kD immunostaining in heat stress. (*a*) Many nerve cells (blank arrows) exhibit upregulation of HSP in the occipital cortex; (*b*) HSP positive glia cells, probably astrocyte around one microvessel can be seen in thalamus of heat stressed rats as well; (*c*) Negative control staining from the occipital cortex of one heat stressed rat showing no positive cell reaction (bar = 80 μm), from Sharma *et al*.[20,95], Sharma[12].

In some neurons, immunostaining of the cell nucleus is quite pronounced (figure 3(B)).

Similarly, a marked increase in HO-2 immunostaining is seen in the brain stem, hypothalamus, thalamus and in the cerebellum. The HO-2 immunoreactivity is often seen in the cell cytoplasm and in many cases the cell nucleus and the karyoplasm remain unstained[95] (figure 3(B)).

7.2.4.5.2. *Ultrastructural localization of nNOS and HO-2 immunoreaction*. NOS and HO immunoreactivity are mainly confined within the cytoplasm of the neurons and in dendrites[83]. These ultrastructural evidence are in line with the biochemical studies showing localization of NOS and HO enzymes at the membrane levels in neuronal cytoplasm attached to the endoplasmic reticulum.

7.2.4.6. *Heat shock protein expression*. The magnitude of cellular stress following heat exposure was examined using immunoreactivity of the inducible isoform of the heat shock protein (HSP, 72 kDa), in the CNS[116,117] using immunohistochemistry. The sub-cellular localization of the HSP was examined at the ultrastructural level.

HSPs are a highly conserved group of proteins that respond to a number of stressors including heat stress and are thought to play a role in cellular repair and in the induction of thermotolerance[116–120]. This idea is further supported by the observations of King *et al.*[122], in mice which showed an increased production of HSP 70 min the liver following WBH and is associated with thermotolerance (see below). The CNS of normal rats did not show any evidence of HSP immunoreactivity. However, after 4 h of heat exposure, a marked increase in the HSP immunostaining was observed in neurons located in several brains and spinal cord regions[18,91] (figure 3). Ultrastructural investigation demonstrated dark reaction product of HSP in dendrites and cytoplasm of nerve cell bodies[91,106]. This observation is in line with other workers in rats[120] and in dogs[121]. There are reasons to beleive that HSPs induced protection of the cells is related to their functions as molecular chaperones[117,120,121]. These molecular chaperones will bind to partially folded or misfolded proteins and, thus, preventing their irreversible denaturation[14,117].

This induction of HSP response in heat stress is no longer evident in rats pretreated with drugs which attenuated the BBB disruption and minimized the cell damage. These observations suggest that induction of HSP response is closely associated with the magnitude of cell injury and are in line with the hypothesis that reduction in cellular stress and/or BBB permeability will attenuate the HSP response[20,103,118,119].

7.2.5. *Gene expression*. Brain injury occurring following trauma, ischemia or hypoxia induces alterations in several gene expression[123]. However, to the authors' knowledge, gene expression studies in heat stress are not yet performed. Preliminary observations from the laboratory suggest that the expression of c-*fos*, c-*jun* and c-*myc* are altered in several brain regions following heat stress (see Sharma *et al.*[11,102]). Thus, an upregulation of c-*fos* is seen in the cortex, hippocampus, thalamus, hypothalamus and spinal cord of 4 h heat stressed rats (Sharma, unpublished observations) compared to normal animals. Upregulation of c-*fos* is seen in layers III–V in the cerebral cortex; CA1-CA3 sub-fields in the hippocampus; dorsal horn of the spinal cord and in several regions of the thalamus and hypothalamus. This indicates that 4 h heat stress is able to activate neurones precisely and

specifically in certain regions of the brain. On the other hand, upregulation of c-*jun* and c-*myc* are not always localized in these regions. The functional significance of these findings in relation to brain pathology is not well understood.

7.2.6. *Apoptosis*. Apoptosis is one of the major mechanisms of cell death which occurs during the developmental process. During secondary brain injury caused by trauma, ischemia, hypoxia and in many other neurodegenarative diseases, apoptotic mechanisms are reactivated to induce cell death[11]. It seems likely that apoptosis plays major roles in cell injury occurring in hyperthermia. However, to the authors' knowledge, apoptotic changes in the CNS following heat injury is not examined yet. The preliminary observations of BCl_2, p^{53} and p^{75} expression in several brain regions indicate that apoptosis plays an important role in heat stress induced cell injury and cell death (Sharma, unpublished observations).

7.2.7. *Expression of NMDRA receptor sub-units*. Increased glutamate concentration in hippocampus prompted one to determine expression of glutamate receptor complexes using northern blot studies on the different sub-units of NMDRA expression. In the hippocampus, downregulation of NMDRA1, NMDRA2 sub-units were seen[124]. A decrease in receptor sub-units may reduce the chances of glutamate induced cell death in the hippocampus. Further studies using glutamate receptor blockers in heat stress are needed to understand the involvement of excitotoxicity in heat induced brain damage.

7.3. *Heat induced brain dysfunction: stress component vs passive heating*

These observations demonstrate that acute heat exposure has the capacity to induce hyperthermia, breakdown of the BBB permeability and brain oedema formation. However, it is not clear whether the neurological symptoms following heat exposure is due to stress associated with heat or due to the effect of passive heating alone (table 4).

To answer this question, rats were exposed to 4 h heat stress at 38°C under urethane or equithesin anaesthesia[20, 80] (Sharma, unpublished observations) (figure 1(*e*)). The anaesthetized rats did not develop stress symptoms (figure 1(*e*)) and in these animals disturbances of the BBB permeability, brain oedema, CBF or 5-HT levels were not present (figure 1(*e*)). There was no difference in the results obtained in rats either anaesthetized with urethane or equithesin. These observations suggest that stress associated with heat is the key factor in underlying brain dysfunction. Obviously, due to a reduction in the perception of stress, anaesthetic compounds apparently offer a certain degree of neuroprotection[47], a subject which encourages additional investigation.

7.4. *Effect of age on heat stress induced brain pathology*

Age is another important factor influencing stress response[125], probably by desensitisation of several neurochemical receptors in the CNS[47]. The influence of age on heat stress induced alterations was examined in the BBB permeability, serotonin metabolism, CBF, brain oedema and cell changes in a separate group of adult rats (age 24–32 weeks)[20,78].

It was found that, in adult rats, heat stress induced changes in the BBB permeability, brain oedema and cell changes were considerably reduced[78] (table 4). The plasma and brain serotonin levels correlated very well with the breakdown of the

Table 4. Effect of age and anaesthesia on the BBB permeability, brain oedema, cerebral blood flow and 5-HT level in control and heat stressed young (age 8–9 weeks) animals. Animals were exposed to heat stress in a BOD incubator at 38°C (rel humid 45–47%, wind vel 20–28 cm/s).

	BBB permeability[a]		Brain oedema[a]		CBF[a]	5-HT level	
Type of experiment	EBA mg %	^{131}I-sodium %	Water content %	Volume swelling %f [b]	ml/g/min µg/g	Brain[a] µg/ml	Plasma
Control (10)	0.28 ± 0.06	0.42 ± 0.08	78.74 ± 0.08	nil	1.04 ± 0.08	0.84 ± 0.08	0.28 ± 0.
Heat stress 4 h (10)	1.14 ± 0.45*	2.20 ± 0.21*	81.45 ± 0.34*	+ 14	0.74 ± 0.05*	2.24 ± 0.31*	1.35 ± 0.12
Heat stress 2 h (5)	0.34 ± 0.11	0.38 ± 0.12	78.87 ± 0.32	nil	0.90 ± 0.08	0.62 ± 0.12	0.34 ± 0.08
Heat stress 1 h (5)	0.38 ± 0.14	0.42 ± 0.21	78.32 ± 0.23	nil	0.98 ± 0.04	0.78 ± 0.14	0.30 ± 0.10
Heat stress 4 h[c] (10)	0.34 ± 0.12	0.48 ± 0.14	78.87 ± 0.12	nil	0.96 ± 0.04	1.24 ± 0.32	0.56 ± 0.2
Heat stress 4 h[d] (5)	0.30 ± 0.12	0.38 ± 0.14	78.35 ± 0.10	nil	0.88 ± 0.06	0.76 ± 0.21	0.34 ± 0.12

* $p < 0.01$, Unpaired student's t-test; [a] = whole brain; [b] = calculated from difference in the water content according to Elliott and Jasper[97]; [c] = adult animals (20–25 weeks old); [d] = Urethane (1.5 g/kg, ip) anaesthetised; Data from Sharma *et al.*[20], Sharma[12].

BBB permeability, brain oedema and cell changes in adult animals. Thus, the adult rats showed only minor changes in the serotonin content in plasma (141%) and brain (20%) (see table 4) indicating that serotonin may be one of the important neurotransmitters involved in the pathophysiology of brain function in hyperthermia[78].

It appears that the intensity of stress, its perception at the cellular level and elicitation of stress response will decrease with advancing age[125]. Another possibility would be that the processing of thermoregulatory information in the CNS at thermoregulatory effectors are more efficient in adult animals compared to young rats (see Sharma and Westman[11]).

7.5. *Influence of heat acclimatization on CNS function*

Recent reports suggest that successive increments in the level of work performed in a hot environment results in heat adaptation[14,15,20]. This heat acclimatization or heat adaptation will allow a person to work safely at levels of heat that were previously intolerable or life-theratening[14,15,20]. This process of heat acclimatization usually takes several days to weeks and probably involves alterations in peripheral or central neurochemical metabolism (for review see Sharma *et al.*[20]). To study the effects of heat acclimation in WBH induced changes in CNS function, the two different sets of animal experiments described below were performed.

7.5.1. *Influence of repeated short-term heat exposure*. To examine the effect of heat adaptation on the consequences of hyperthermic brain injury, a separate group of rats were exposed to 1 or 2 h heat stress daily at 38°C for 7 days. On the 8th day, these rats were subjected to 4 h heat stress. The results showed that subjection of rats to 1 or 2 h HS daily for 7 days did not result in symptoms[77]. Their rectal temperature and plasma and brain serotonin levels were slightly but significantly elevated on the 7th day compared to the *normal* rats kept at room temperature (21 ± 1°C). Heat adapted rats when given an additional 4 h heat stress on the 8th day did not elicit any further rise in either the rectal temperature or the serotonin level and the breakdown of the BBB permeability to Evans blue and ^{131}I-sodium tracers was not observed[77]. The brain water content was within the normal range. Morphological examination showed almost normal appearance of the neuropil[20] (figure 2(C.*g*)).

These findings suggest that a slight but significant increase in the basal serotonin level and rectal temperature following animals subjected to repeated heat stress induces heat tolerance[77]. Obviously in the absence of BBB breakdown and brain oedema formation, the cell changes are considerably reduced following heat stress. This suggests that the BBB breakdown in heat stress is instrumental in precipitating brain oedema formation and cell injury[20]. Similarly, pre-conditioning of mice by exposing them to short periods of WBH (30 min) resulted in a significant increase in the liver HSP[122]. Subjection of these mice to a lethal heat stress at 41°C 48 h after recovery resulted in a more than 88% survival rate[112]. These studies suggest that thermotolerance caused by repeated heat exposure attenuates heat toxicity in animals probably via an enhanced production of HSP[122].

7.5.2. *Influence of rearing at high ambient temperature*. To further confirm the above hypothesis, the influence was examined of rearing at high ambient temperatures on the consequences of heat stress induced changes in the BBB permeability, brain oedema formation and cell injury in separate group of rats[74]. Separate

groups of rats were placed immediately after weaning (on day 21) at warm ($28 \pm 1^{\circ}C$) or hot ($34 \pm 1^{\circ}C$) ambient air temperatures respectively for 6 weeks. The control group of rats were kept at normal room temperature ($21 \pm 1^{\circ}C$). At the age of 9 weeks, these rats were exposed to 4 h heat stress at 38°C and development of stress symptoms, BBB permeability, brain oedema and cell changes were examined[74].

In these heat adapted rats only mild stress symptoms were seen, whereas rats reared at hot ambient temperature did not show any symptoms. Extravasation of tracers across the BBB or increase in the brain water content were absent, and the decline in the CBF was limited to only 12%. The basal level of plasma and brain serotonin levels were mildly elevated in these rats that did not increase further following additional heat exposure. No distortion of nerve cells, glial cells or myelin damage seen in the heat adapted animals at both light and electron microscopy[74], indicating that BBB breakdown is instrumental in inducing cell injury.

The probable mechanisms of such heat adaptation at the cellular or molecular level in the CNS are not well understood. A possibility exists that exposure to heat stimulus will induce upregulation of heat shock proteins in the CNS, which could play an important neuroprotective role.

These observations clearly suggest that pre-conditioning of animals to mild or moderate heat for short- or long-term periods induce acclimatization. These animals when subjected to WBH did not result in symptoms or encephalopathy.

8. Conclusion

In conclusion, studies in several animal models and in humans suggest that heat can directly induce nervous tissue injury. The severity of the injury depends on the level and duration of heating. Observations in selected groups of patients with cancer, who received WBH as treatment, suggest that the critical thermal maximum temperature is between 41.6–42°C for 45 min to 8 h. At extreme temperatures of 49–50°C in animal studies, all cellular structures are destroyed and cell death can be seen within 5 min. Animal studies further show that hyperthermia ($>41^{\circ}C$) in rats is associated with endothelial cell injury accompanied by breakdown of the BBB that results in profound oedema and cell damage. This increase in the BBB permeability is mediated by several neurochemicals. This effect of hyperthermia on brain dysfunction is reduced by advancing age, anaesthesia or prior heat experience. This indicates that the age and physiological states of the animals prior to heat exposure are important determining factors for the outcome of thermal brain damage.

8.1. *Significance of the findings*

Experimental observations on hyperthermia induced brain oedema, and cell changes have added a new dimension in the field of heat stress and CNS injury. The results show that brain oedema formation can occur in heat stress due to breakdown of the BBB, a feature which is quite comparable to that of traumatic brain oedema. This breakdown of the BBB in hyperthermia can be prevented by many pharmacological agents, either interfering with the stress mechanisms or influencing the metabolism of various neurochemical mediators. Thus, these findings using a new approach of hyperthermic brain injury suggest that the mechanisms underlying cellular injury in the CNS following various types of insults such as ischemia, hypoxia, trauma, etc. are quite similar in nature. The magnitude and severity of the primary insult to the CNS mainly determine the final outcome of cell injury in

a particular situation. Another important point has emerged from this study that in any pathological condition, no single chemical compound or factor is responsible for all the neuropathological changes seen in the CNS. In fact, the final outcome is due to a combination of many factors and neurochemicals exerting a synergistic influence on the CNS. Thus, to achieve a goal of neuroprotection, these multiple factors must be considered in order to develop a suitable therapeutic strategy to treat the problems of CNS injury.

9. Future perspectives

In order to understand the molecular mechanisms of heat induced cell injury in great detail, further investigations on additional factors involved in cell damage and repair mechanisms are needed using animal models.

Acknowledgements

Authors research reported in this review is supported by grants from Swedish Medical Research Council nr. 2710 (JW/HSS), Göran Gustafsson Foundation (HSS), Stockholm, Sweden, Astra Hässle (HSS), Mölndal, Sweden; Alexander von Humboldt Foundation (HSS), Bonn, Germany, Dr Wilmar Schwabe (HSS) GmbH, Karlsruhe, Germany; IPSEN-Beufour (HSS), Paris, France. The technical assistance of Kärstin Flink, Ingmarie Olsson, Franziska Drum, and secretarial assistance of Aruna Sharma is greatly appreciated. We thank the anonymous reviewer for his excellent suggestions on this review for better understanding.

References

1. Gauss H, Meyer KA. Heat stroke: report of one hundred and fifty-eight cases from Cook County Hospital, Chicago. *Am J Med Sci* 1917; 154: 554–64.
2. Weisenburg TH. Nervous symptoms following sunstroke. *JAMA* 1912; 58: 2015–7.
3. Malamud N, Haymaker W, Custer RP. Heat stroke. A clinicopathological study of 125 fatal cases. *Milit Surg* 1964; 99: 397–449.
4. Sterner S. Summer heat illness. *Postgrad Med* 1990; 87: 67–73.
5. Hahn GM. *Hyperthermia and Cancer*, New York: Plenum Press, 1982.
6. Hoopes PJ. The effects of heat on the nervous system. In: Gutin PH, Leibel SA, Sheline GE, eds, *Radiation Injury to the Nervous System*, New York: Raven Press; 1991; 407–30.
7. Ryan TP, Trembly BS, Roberts DW, Strohbehn JW, Coughlin CT, Hoopes PJ. Brain hyperthermia I: interstitial microwave antenna array techniques, the Dartmouth experience. *Int J Radiat Oncol Biol Phys* 1994; 29: 1065–78.
8. Hartman FW, Major RC. Pathological changes resulting from accurately controlled artificial fever. *Am J Clin Path* 1935; 5: 392–410.
9. Alpers BJ. Hyperthermia due to lesions in the hypothalamus. *Arch Neurol & Psychiat* 1936; 35: 30–42.
10. Siesjö BK. *Brain Energy Metabolism*, New York, Chichester: Wiley, 1978.
11. Sharma HS, Westman J. Brain functions in hot environment. *Prog Brain Res* 1998; 115: 1–527.
12. Sharma HS. Pathophysiology of blood–brain barrier, brain edema and cell injury following hyperthermia: new role of heat shock protein, nitric oxide and carbon monoxide. An experimental study in the rat using light and electron microscopy. *Acta Universitatis Upsaliensis* 1999; 830: 1–94.
13. Sharma HS, Westman J. Pathophysiology of hyperthermic brain injury. Current concepts, molecular mechanisms and pharmacological strategies. In: Oehnmichen, ed., *Research in Legal Medicine Vol. 21 Hyperthermia, Burning and Carbon Monoxide*, Lübeck Medical University Publications, Lübeck, Germany: Schmidt-Römhild Verlag, 2000; 79–120.
14. Bouchama A, Knochel JP. Heat stroke. *N Engl J Med* 2002; 25: 1978–88.

15. Knochel JP, Reed G. Disorders of heat regulation. In: Narins RG, ed., *Maxwell & Kleeman's clinical disorders of fluid and electrolyte metabolism*, 5th edn, New York: McGraw-Hill, 1994; 1549–90.
16. Milton AS. *Physiology of Thermoregulation*, Basel: Birkhauser, 1994.
17. Blatteis C. Thermoregulation: recent progress and New Frontiers. *Ann. NY Acad Sci* 1997; 813: 1–865.
18. Ellis FP. Mortality from heat illness and heat-aggravated illness in the United States. *Environ Res* 1972; 5: 1–58.
19. Austin MG, Berry JW. Observation on one hundred cases of heatstroke. *JAMA* 1956; 161: 1525–9.
20. Sharma HS, Westman J, Nyberg F. Pathophysiology of brain edema and cell changes following hyperthermic brain injury. Brain functions in hot environment. *Prog Brain Res* 1998; 115: 351–412.
21. Cervós-Navarro J, Ferszt R. Brain edema: pathology, diagnosis and therapy. *Adv Neurol*, New York: Raven Press, 1980; 1–450.
22. Go KG, Baethmann A. *Recent Progress in the Study and Therapy of Brain Edema*, New York: Plennum Press, 1984.
23. Wahl M, Unterberg A, Baethmann A, Schilling L. Mediators of blood–brain barrier dysfunction and formation of vasogenic brain edema. *J Cereb Blood Flow Metab*, 1988; 8: 621–34.
24. Sharabi Y, Danon YL, Berkenstadt H, Almog S, Mimouni-Bloch A, Zisman A, Dani S, Atsmon J. Survey of symptoms following intake of pyridostigmine during Persian Gulf War. *Isr J Med Sci* 1991; 27: 656–8.
25. Hanin I. The Gulf War, stress and a leaky blood-brain barrier (News & Views). *Nature Med* 1996; 2: 1307–8.
26. Friedman A, Kaufer D, Shemer J, Hendler I, Soreq H, Tur-Kapsa I. Pyridostigmine brain penetration under stress enhances neuronal excitability and induces early immediate transcriptional response. *Nature Med* 1996, 2; 1382–5.
27. Sharma HS, Dey PK. Impairment of blood–brain barrier by immobilization stress: role of serotonin. *Indian J Physiol Pharmacol* 1981; 25: 111–22.
28. Sharma H S. Blood–brain barrier in stress. PhD Thesis, Varanasi (India): Banaras Hindu University, 1982.
29. Sharma HS, Dey PK. Role of 5-HT on increased permeability of blood–brain barrier under heat stress. *Indian J Physiol Pharmacol* 1984; 28: 259–67.
30. Sharma HS, Dey PK. Probable involvement of 5-hydroxytryptamine in increased permeability of blood–brain barrier under heat stress. *Neuropharmacology* 1986; 25: 161–7.
31. Sharma HS, Dey PK. Influence of long-term immobilization stress on regional blood–brain barrier permeability, cerebral blood flow and 5-HT level in conscious normotensive young rats. *J Neurolog Sci* 1986; 72: 61–76.
32. Sharma HS, Dey PK. Influence of long-term acute heat exposure on regional blood–brain barrier permeability, cerebral blood flow and 5-HT level in conscious normotensive young rats. *Brain Res* 1987; 424: 153–62.
33. Sharma HS, Cervós-Navarro J. Brain oedema and cellular changes induced by acute heat stress in young rats. *Acta Neurochir (Wien)* 1990; Suppl. 51: 383–6.
34. Sharma HS, Cervós-Navarro J, Dey PK. Increased blood–brain barrier permeability following acute short-term forced-swimming exercise in conscious normotensive young rats. *Neurosci Res* 1991; 10: 211–21.
35. Hart GR, Anderson RJ, Crumpler CP, Shulkin A, Reed G, Knochel JP. Epidemic classical heat stroke: clinical characteristics and course of 28 patients. *Medicine* 1982; 61: 189–97.
36. Knochel JP. Environmental heat illness. An eclectic review. *Arch Intern Med* 1974; 133: 841–64.
37. Zimmerman HM. Temperature disturbances and the hypothalamus. A Research. *Nerv Ment Dis Proc* 1940; 20: 824–40.
38. Kapp DS and Lord PF. Thermal tolerance to whole body hyperthermia. *Int J Radiat Oncol Biol Phys* 1983; 9: 917–21.
39. Bull JMC. An update on the anticancer effects of a combination of chemotherapy and hyperthermia. *Cancer Res* 1984; 44: 4853–4875.

40. Ryan TP, Hoopes PJ, Taylor JH, Strohbehn JW, Roberts DW, Double EB, Coughlin CT. Experimental brain hyperthermia: techniques for heat delivery and thermometry. *Int J Radiat Oncol Biol Phys* 1991; 20: 739–50.
41. Ryan TP, Wykoff R, Hoopes PJ. An automated temperature mapping system for use in ultrasound or microwave hyperthermia. *J Biomed Eng* 1991; 13: 348–54.
42. Sminia P, van der Zee J, Wondergem J, Haveman J. Effect of hyperthermia on the central nervous system: a review. *Int J Hyperthermia* 1994; 10: 1–30.
43. van der Zee J. Whole body hyperthermia. The development of and experience with a clinical method. Dissertation, Rotterdam (Netherlands): Erasmus University, 1987.
44. Kerner T, Deja M, Ahlers O, Hildebrandt B, Dieing A, Riess H, Wust P, Gerlach H. *Acta Anaesthesiol Scand* 2002; 46: 561–6.
45. Tanaka R, Kim CH, Yamada N, Saito Y. Radiofrequency hyperthermia for malignant brain tumours: preliminary results of clinical trials. *Neurosurgery* 1987; 21: 478–83.
46. Petrovich Z, Langholz B, Gibbs FA, Sapozink MD, Krapp DS, Stewart RJ, Emami B, Oleson J, Senzer N, Slater J, Astrahan M. *Int J Radiat Oncol (Biol Phys)* 1989 Mar; 16 (3): 601–7.
47. Salzman SK. *Neural Monitoring. The Prevention of Intraoperative Injury*, New Jersey: Humanna Press, 1990.
48. Mellergard P, Nordström CH. Epidural temperature and possible intracerebral temperature gradients in man. *Br J Neurosurg* 1990; 4: 31–8.
49. Cabanac M. Selective brain cooling and thermoregulatory set-point. *J Basic Clin Physiol Pharmacol* 1998; 9: 3–13.
50. Gabay C, Kushner I. Acute phase proteins and other systemic response to inflammation. *N Engl J Med* 1999; 340: 448–54 [Erratum, 340: 1376].
51. Chang DM. The role of cytokines in heatstroke. *Immunol Invest* 1993; 22: 553–61.
52. Katschinski DM, Wiedemann GJ, Longo W, d'Oleire FR, Spriggs D, Robbins HI. Whole body hyperthermia cytokine induction: a review, and unifying hypothesis for myeloprotection in the setting of cytotoxic therapy. *Cytokine Growth Factor Rev* 1999; 10: 93–97.
53. Pedersen BK, Hoffman-Goetz L. Exercise and the immune system: regulation, integration, and adaptation. *Physiol Rev* 2000; 80: 1055–81.
54. Kraybill WG, Olenki T, Evans SS, Ostberg JR, O'Leary KA, Gibbs JF, Repasky EA. A phase I study of fever-range whole body hyperthermia (FR-WBH) in patients with advances solid tumours:correlation with mouse models. *Int J Hyperthermia* 2002; 18: 253–66.
55. Gottschalk PG, Thomas JE. Heat stroke. *Mayo Clin Proc* 1966; 41: 470–82.
56. Frankel HM, Ellis JP Jr, Cain SM. Development of tissue hypoxia during progressive hyperthermia in dogs. *Am J Physiol* 1963; 205: 733–7.
57. Shibolet S, Coll R, Gilat T, Sohar E. Heatstroke: its clinical picture and mechanisms in 36 cases. *Quart J Med* 1967; 36: 525–48.
58. Shapiro Y, Rosenthal T, Sohar E. Experimental heatstroke. A model in dogs. *Arch Int Med* 1973; 131: 688–92.
59. Chapot G, Muller M, Barrault N, Dargnat N, Languillat J-M. A correlation between $PaCO_2$ and body temperature in febrile patients. *Thorax* 1974; 29: 104–5.
60. Nemoto EM, Frankel HM. Cerebral oxygenation and metabolism during progressive hyperthermia. *Am J Physiol* 1970; l219: 1784–8.
61. Field J (II), Fuhrman FA, Martin AW. Effect of temperature on the oxygen consumption of brain tissue. *J Neurophysiol* 1944; 7: 117–26.
62. Burger FJ, Fuhrman FA. Evidence of injury by heat in mammalian tissues. *Am J Physiol* 1964; 206: 1057–61.
63. Heyman A, Patterson JL Jr, Nichols FT Jr. The effects of induced fever on cerebral functions in neurosyphlis. *J Clin Invest* 1950; 29: 1335–41.
64. Demers HG, Spaich P, Usinger W. Der Hirnkreislauf bei erhöhter Körper-temperature. *Verh Dtsch ges Kreislaufforschg* 1969; 35: 131–40.
65. Reinmuth OM, Scheinberg P, Bourne B. Total cerebral blood flow and metabolism. *Arch Neurol (Chic)* 1965; 12: 49–66.
66. Hales JRS, Findlay JD, Mabon RM. Tissue hypoxia in oxen exposed to severe heat. *Respir Physiol* 1967; 3: 43–6.

67. Carlsson C, Hägerdal M, Siesjö BK. The effect of hyperthermia upon oxygen consumption and blood flow in the cerebral cortex of the rat. *Acta Anaesth Scand* 1976; 26: 1001–6.
68. Thrall DE, Prescott DM, Samulski TV, Dewhirst MW, Cline JM, Lee J, Page RL, Oleson JR. Serious toxicity associated with annular microwave array induction of whole-body hyperthermia in normal dogs. *Int J Hyperthermia* 1992; 8: 23–32.
69. Britt RH, Lyons BE, Ryan T, Saxer E, Obana W, Rossi G. Effect of whole body hyperthermia on auditory brainstem and somatosensory and visualevoked potentials. In: Hales RRS, ed., *Thermal Physiology*, New York: Raven Press, 1984; 519–23.
70. Marsh RR, Yamane H, Potsic WP. Auditory brain-stem response and temperature. Relationship in the guinea pig. *Electroencephalog Clin Neurophysiol* 1984; 57: 289–93.
71. Sharma HS, Dey PK, Kumar A. Role of circulating 5-HT and lung MAO activity in physiological processes of heat adaptation in conscious young rats. *Biomedicine* 1986; 6: 31–40.
72. Sharma HS, Cervós-Navarro J. Nimodipine improves cerebral blood flow and reduces brain edema, cellular damage and blood-brain barrier permeability following heat stress in young rats. In: Krieglstein J, Oberpichler H, eds, *Pharmacology of Cerebral Ischemia*, Boca Raton, FL: CRC Press), 1990; 303–10.
73. Sharma HS, Cervós-Navarro J, Dey PK. Acute heat exposure causes cellular alteration in cerebral cortex of young rats. *NeuroReport* 1991; 2: 155–8.
74. Sharma HS, Cervós-Navarro J, Dey PK. Rearing at high ambient temperature during later phase of the brain development enhances functional plasticity of the CNS and induces tolerance to heat stress. An experimental study in the conscious normotensive young rats. *Brain Dysfunction* 1991; 4: 104–24.
75. Sharma HS, Zimmer C, Westman J, Cervós-Navarro J. Acute systemic heat stress increases glial fibrillary acidic protein immunoreactivity in brain. An experimental study in the conscious normotensive young rats. *Neuroscience* 1992; 48: 889–901.
76. Sharma HS, Kretzschmar R, Cervós-Navarro J, Ermisch A, Rühle H-J, Dey PK. Age-related pathophysiology of the blood–brain barrier in heat stress. *Prog Brain Res* 1992; 91: 189–96.
77. Sharma HS, Westman J, Nyberg F, Cervós-Navarro J, Dey PK. Role of serotonin in heat adaptation: an experimental study in the conscious young rat. *Endocrine Reg* 1992; 26: 133–42.
78. Sharma HS, Nyberg F, Cervós-Navarro J, Dey PK. Role of catecholamines in the pathophysiology of heat stress. *Proc 7th Int Catecholamine Symp*, 22–26 June; Amsterdam, Netherlands, 1992; 287.
79. Sharma HS, Westman J, Nyberg F, Cervós-Navarro J, Dey PK. Role of serotonin and prostaglandins in brain edema induced by heat stress. An experimental study in the rat. *Acta Neurochirurgica (Suppl)* 1994; 60: 65–70.
80. Sharma HS, Westman J, Nyberg F, Zimmer C, Cervós-Navarro J, Dey PK. Selective vulnerability of rat hippocampus in heat stress. In: Milton AS, ed., *Temperature Regulation. Advances in Pharmacological Science*, Basel: Birkhauser, 1994; 267–72.
81. Sharma HS, Westman J, Cervós-Navarro J, Dey PK, Nyberg F. Probable involvement of serotonin in the increased permeability of the blood–brain barrier by forced swimming. An experimental study using Evans blue and ^{131}I-sodium tracers in the rat. *Behav Brain Res* 1995; 72: 189–96.
82. Sharma HS, Westman J, Cervós-Navarro J, Nyberg F. Role of neurochemicals in brain edema and cell changes following hyperthermic brain injury in the rat. *Acta Neurochir* 1997; (Suppl. 70): 269–74.
83. Sharma HS, Westman J, Alm P, Sjöquist P-Ö, Cervós-Navarro J, Nyberg F. Involvement of nitric oxide in the pathophysiology of acute heat stress in the rat influence of a new antioxidant compound h 290/51. *Ann NY Acad Sci* 1997b; 813: 581–90.
84. Sharma HS, Westman J, Cervós-Navarro J, Dey PK, Nyberg F. Opioid receptor antagonists attenuate heat stress induced reduction in cerebral blood flow, increased blood–brain barrier permeability, vasogenic edema and cell changes in the rat. *Ann NY Acad Sci* 1997; 813: 559–71.
85. Dey S, Dey PK, Sharma HS. Regional metabolism of 5-hydroxytryptamine in brain under acute and chronic heat stress. *Indian J Physiol Pharmacol* 1993; 37: 8–12.

86. Hall WW, Wakefield FG. A study of experimental heatstroke. *JAMA* 1927; 89: 177–82.
87. Morgan LO. Cell changes in some of the hypothalamic nuclei in experimental fever. *J Neurophysiol* 1938; 1: 281–5.
88. Weiner JC. Experimental study of heat collapse. *J Ind Hyg Toxicol* 1938; 20: 389–400.
89. Wilson SJ, Doan CA. The pathogenesis of hemorrhage in artificially induced fever. *Ann Int Med* 1939; 13: 1214–29.
90. Adolph EF. Tolerance to heat and dehydration in several species of mammals. *Am J Physiol* 1947; 151: 564–75.
91. Haubbard RW, Bowers WD, Mather WT, Curtis FC, Criss REL, Sheldon GM, Ratteree JWJ. Rat model of acute heat stroke mortality. *J Appl Physiol* 1977; 42: 807–16.
92. Buolochnik ED, Zyablov MP. Effect of hyperthermia induced by a high ambient temperature on the direct cortical response. *Byulleten 'Eksperimental'noi Biologii i Meditsiny* 1978; 84: 657–60.
93. Lee DHK. Terrestrial animals in dry heat. In: Dill DB, Adolph EF, Wilber CG, eds, *Handbook Physiol, Section IV, Adaptation to the Environment*, Washington: American Physiological Society, 1964; 55–72.
94. Rapoport SI. *Blood–Brain Barrier in Physiology and Medicine*, New York: Raven Press, 1976.
95. Sharma HS, Alm P, Westman J. Nitric oxide and carbon monoxide in the pathophysiology of brain functions in heat stress. *Prog Brain Res* 1998; 115: 297–333.
96. Sharma HS. Bradykinin$_2$ receptor antagonist HOE-140 attenuates blood–spinal cord barrier permeability following trauma to the rat spinal cord. *Acta Neurochir (Wien)* 2000; Suppl. 76: 159–63.
97. Elliott KAC, Jasper H. Measurement of experimentally induced brain swelling and shrinkage. *Am J Physiol* 1949; 157: 122–8.
98. Olesen SP. An electrophysiological study of microvascular permeability and its modulation by chemical mediators. *Acta Physiol Scand* 1989; 136 (Suppl. 579): 1–28.
99. Bradbury MWB. *The Concept of a Blood–Brain Barrier*, London: Chicester, 1979.
100. Black KL. Biochemical opening of the blood–brain barrier. *Adv Drug Del Rev* 1995; 15: 37–52.
101. Toh CC. Effect of temperature on the 5-hydroxytryptamine content of tissues. *J Physiol (Lond)* 1960; 151: 410–5.
102. Sharma HS, Olsson Y, Dey PK. Changes in blood–brain barrier and cerebral blood flow following elevation of circulating serotonin level in anaesthetized rats. *Brain Res* 1990; 517: 215–23.
103. Sharma HS, Nyberg F, Gordh T, Alm P, Westman J. Neurotrophic factors attenuate neuronal nitric oxide synthase upregulation, microvascular permeability disturbances, edema formation and cell injury in the spinal cord following trauma. In: Stålberg E, Sharma HS, Olsson Y, eds, *Spinal Cord Monitoring*, Wien, New York: Springer-Verlag, 1998; 118–48.
104. Westman J, Sharma HS. Heat shock protein response in the CNS following heat stress. *Prog Brain Res* 1998; 115: 207–39.
105. Johansson BB, Owman Ch, Widner H. *Pathophysiology of the blood–brain barrier. Fernström Foundation Series 14*, Amsterdam: Elsevier, 1990: 1–340.
106. Lum H, Malik AB. Regulation of vascular endothelial barrier function. *Am J Physiol* 1944; 267: 223–41.
107. Davson H. *Physiology of the Cerebrospinal Fluid*, London: Churchill, 1967.
108. Brightman MW, Reese TS. Junctions between intimately apposed cell membranes in the vertebrate brain. *J Cell Biol* 1969; 40: 648–77.
109. Driscoll BF, Kramer AJ, Kies MW. Myelin basic protein:Location of multiple independent antigenic regions. *Science* 1974; 184: 73–5.
110. Cervós-Navarro J, Sharma HS, Westman J, Bongcam-Rudloff E. Glial reactions in the central nervous system following heat stress. *Prog Brain Res* 1998; 115: 241–74.
111. Bredt DS, Snyder SH. Nitric oxide, a novel neuronal messenger. *Neuron* 1992; 8: 3–11.
112. Chiueh CC, Gilbert DL, Colton CA. The neurobiology of NO· and ·OH. *Ann NY Acad Sci* 1994; 738: 1–471.

113. Sharma HS, Drieu K, Alm P, Westman J. Upregulation of neuronal nitric oxide synthase, edema and cell injury following heat stress are reduced by pretreatment with EGB-761 in the rat. *J Therm Biol* 1999; 24: 439–46.
114. Sharma HS, Drieu K, Alm P, Westman J. Role of nitric oxide in blood–brain barrier permeability, brain edema and cell damage following hyperthermic brain injury. an experimental study using EGB-761 and Gingkolide B pretreatment in the rat. *Acta Neurochir (Wien)* 2000; Suppl. 76: 81–6.
115. Alm P, Sharma HS, Hedlund S, Sjöquist P-O, Westman J. Nitric oxide in the pathophysiology of hyperthermic brain injury. Influence of a new anti-oxidant compound H-290/51. *Amino Acids* 1998; 14: 95–104
116. Lindquist S. The heat-shock response. *Ann Rev Biochem* 1986; 55: 1151–91.
117. Welch WJ. Mammalian stress response: cell physiology, structure/function of stress proteins, and implications for medicine and diseases. *Physiol Rev* 1992; 72: 1063–81.
118. Westman J, Drieu K, Sharma HS. Antioxidant compounds EGB-761 and BN-520 21 attenuate heat shock protein (HSP 72 kD) response, edema and cell changes following hyperthermic brain injury. An experimental study using immunohistochemistry in the rat. *AminoAcids* 2000; 19: 339–50.
119. Hedlund S, Sharma HS, Sjöquist P-O, Westman J. A new antioxidant compound h-290/51 attenuates heat shock protein (HSP 72 kD) response, breakdown of the blood–brain barrier permeability, edema and cell injury following acute heat exposure. An experimental study using light and electron microscopy in the rat. *J Therm Biol* 1999; 24: 409–14.
120. Walters TJ, Ryan KL, Mason PA. Regional distribution of Hsp 70 in the CNS of young and old food-restricted rats following hyperthermia. *Brain Res Bull* 2001; 55: 367–74.
121. Oglesbee MJ, Alldinger S, Vasconcelos D, Diehl KA, Shinko PD, Baumgärtner W, Tallman R, Podell M. Intrinsic thermal resistance of the canine brain. *Neuroscience* 2002; 113: 55–64.
122. King YT, Lin CS, Lin JH, Lee WC. Whole body hyperthermia induced thermotolerance is associated with the induction of heat shock protein 70 in mice. *J Exp Biol* 2002; 205: 273–8.
123. Hughes P, Dragunow M. Induction of immediate-early genes and the control of neurotransmitter-regulated gene expression within the nervous system. *Pharmacol Rev* 1995; 47: 133–78.
124. Le Grevés P, Sharma HS, Westman J, Alm P, Nyberg F. Acute heat stress induced edema and nitric oxide synthase upregulation and downregulates mRNA levels of the NMDAR1, MNDAR2 and NMDAR2B subunits in the rat hippocampus. *Acta Neurochir* 1997; Suppl. 70: 275–8.
125. Selye H. *Stress in Health and Disease*, London: Butterworths, 1976.