Acta Neurochir (2006) [Suppl] 96: 426–431

Printed in Austria

Whole-body hyperthermia in the rat disrupts the blood-cerebrospinal fluid barrier and induces brain edema

H. S. Sharma[1], **J. A. Duncan**[2], and **C. E. Johanson**[2]

[1] Laboratory of Cerebrovascular Research, Institute of Surgical Sciences, Department of Anesthesiology & Intensive Care, University Hospital, Uppsala University, Uppsala, Sweden
[2] Department of Neurosurgery, Brown Medical School, Rhode Island Hospital, Providence, RI, USA

Summary

The present investigation was undertaken to find out whether whole-body hyperthermia (WBH) alters blood-cerebrospinal fluid barrier (BCSFB) permeability to exogenously-administered tracers and whether choroid plexus and ependymal cells exhibit morphological alterations in hyperthermia. Rats subjected to 4 hours of heat stress at 38 °C in a biological oxygen demand (BOD) incubator exhibited a profound increase in the BCSFB to Evans blue and radioiodine. Blue staining of the dorsal surface of the hippocampus and caudate nucleus and a significant increase in Evans blue and $^{[131]}$Iodine in cisternal cerebrospinal fluid were seen following 4-hour heat stress compared to control. Degeneration of choroidal epithelial cells and underlying ependyma, a dilated ventricular space, and degenerative changes in the underlying neuropil were frequent. Hippocampus, caudate nucleus, thalamus, and hypothalamus exhibited profound increases in water content after 4 hours of heat stress. These observations suggest that hyperthermia induced by WBH is capable of breaking down the BCSFB and contributing to cell and tissue injury in the central nervous system.

Keywords: Hyperthermia; blood-cerebrospinal fluid barrier; edema.

Introduction

Hyperthermia and heat-related illnesses cause large numbers of deaths (about 800 to 2000 cases) during summer months in the United States and in Europe [1, 2, 7–9]. Heat-related death far exceeds that of any other natural calamity such as floods, cyclones, or hurricanes [1, 3, 13, 18, 20, 21, 25, 42, 43]. Recently, the number of heat stroke-induced deaths have increased with global warming and with world-wide increase in the frequency and intensity of heat waves [2, 18, 27, 29, 35, 36].

Heat stress and associated heat stroke are life-threatening illnesses in which body temperature increases above 40 °C causing severe central nervous system (CNS) dysfunction, such as delirium, convulsion, and coma [1–3]. More than 50% of heat stroke victims die within a short time, despite lowering of the body temperature and therapeutic intervention [2, 7–9]. Those who survive heat stroke often show permanent neurological deficit [2, 4, 21, 25].

Interestingly, whole-body hyperthermia (WBH) is commonly used as an adjunct to cytotoxic therapy for cancer [14, 15, 19, 36, 44]. Recently it has been recognized that WBH combined with cytotoxic therapy for cancer causes inhibition of DNA repair, increased drug permeation, and decreased resistance to DNA damaging agents [24]. New clinical and experimental results show that WBH enhances cytotoxic ionizing radiation and chemotherapy [10, 14, 15, 24, 36]. There are reasons to believe that WBH-induced severe side effects, including altered brain function, are probably due to breakdown of BBB function [28, 29, 31].

Previous experiments on WBH in our laboratory suggest that alterations in the brain fluid microenvironment following heat stress are responsible for hyperthermia-induced brain damage [26, 32–34, 38]. However, studies on alterations in the blood-cerebrospinal fluid barrier (BCSFB) in heat stress are still lacking. The BCSFB maintains the composition of the CSF and regulates homeostasis of the CNS within a strict normal limit [6, 16, 22]. Thus, breakdown of the BCSFB adversely influences CNS structure and function.

The present investigation was undertaken to find

out whether WBH alters BCSFB permeability to exogenously-administered tracers and whether choroid plexus and ependymal cells exhibit morphological alterations during hyperthermia.

Materials and methods

Animals

Experiments were carried out on male Sprague-Dawley rats (100 to 150 g; aged 12 to 16 weeks) housed at a controlled room temperature (21 ± 1 °C) on a 12-hour light, 12-hour dark schedule. Food and tap water were supplied ad libitum before the experiments.

Whole-body hyperthermia

Rats were exposed to WBH in a biological oxygen demand incubator (relative humidity 45 to 50%; wind velocity 18 to 25 cm/sec) maintained at 38 °C for 1 to 4 hours [26, 30, 32, 33]. The experiments were conducted according to National Institutes of Health (USA) guidelines for use and care of animals [26, 27, 30]. This experiment was approved by the Ethics Committee of Uppsala University.

BCSFB permeability

The BCSFB was examined in vivo using Evans blue (2%, 0.3 mL/100 g) and $^{[131]}$Iodine (10 μCi/100 g) tracers [30, 31, 37, 39, 40]. The tracers were administered into the right femoral artery and allowed to circulate for 5 minutes. At the end of the experiment, a CSF sample (about 100 μl) was drawn from the cisterna magna without blood contamination. The animals were then perfused with 0.9% saline through the heart and the brain was dissected out. Extravasation of Evans blue dye was visually examined in the ventricular walls of the lateral, third, and fourth ventricles. Various parts of the brain were then dissected out, weighed, and the radioactivity counted in a gamma counter (energy window 500–800 keV) [30]. Before perfusion, a whole blood sample was withdrawn from the left ventricle by cardiac puncture and the radioactivity determined as above [26, 30, 32]. Extravasation of tracers into the CSF as well as other brain areas around the ventricular system was expressed as percentage increase over the whole blood radioactivity [30]. Evans blue dye that entered some areas of the brain was also measured colorimetrically [26, 30–32].

Brain edema

Brain edema formation was measured using water content calculated from the difference between wet and dry weights of the samples, either in the whole brain or in the several identical brain regions used for radiotracer measurement, as described above [30].

Morphological investigations

At the end of the experiments, rats were perfused transcardially with 4% paraformaldehyde in 0.1 mol phosphate buffer (pH 7.4), preceded by a brief saline rinse [37, 38]. The animals were wrapped in aluminum foil and kept at 4 °C overnight. On the next day, the brain and spinal cord were dissected out and small pieces were embedded in paraffin. About 3 μm thick sections were cut and stained with hematoxylin and eosin or Nissl and examined under a bright field microscope (Leica Microsystems, Bannockburn, IL) for neurodegenerative changes [27]. For semiquantitative analyses of cell injury, rough scores of 0 (no damage), or 1 (least damage) to 4 (maximum damage) were assigned in a blinded fashion [41].

Statistical analyses

Quantitative data were analyzed using ANOVA followed by Dunnet's test for multiple group comparison. The semiquantitative data were analyzed with the chi-square test.

Results

BCSFB permeability

Rats subjected to 4 hours of WBH at 38 °C in a biological oxygen demand incubator exhibited profound alterations in BCSFB to Evans blue and radioiodine tracers. Mild to moderate blue staining of the walls in the lateral, third, and fourth cerebral ventricles was noted. The dorsal surface of the hippocampus and caudate nucleus showed moderate staining. The choroid plexus had deep blue staining. Measurement of Evans blue dye in selected brain regions, such as the hippocampus, caudate nucleus, mid-thalamus (massa intermedia), hypothalamus, dorsal surface of the brain stem, and ventral surface of the cerebellum showed a significant increase compared to the control group (Table 1). A significant increase in Evans blue and $^{[131]}$Iodine tracers was observed in the CSF samples obtained from the cisterna magna following 4 hours of WBH compared to the control group (Table 1).

On the other hand, blue staining of the ventricular walls and/or surface of the structures within the cerebral ventricles was absent in animals subjected to 1 or 2 hours of heat stress. At these earlier times, no significant increase in Evans blue or radioiodine tracer was noted in various brain areas and/or CSF samples (Table 1).

Brain edema

Measurement of water content in identical brain regions showing leakage of Evans blue or radiotracers, e.g., hippocampus, caudate nucleus, mid-thalamus (massa intermedia), hypothalamus, dorsal surface of the brain stem, and the ventral surface of the cerebellum (sample size 130 to 220 mg wet weight), exhibited a significant increase in water content after 4 hours of WBH (Table 1). However, rats subjected to 1 or 2 hours of heat exposure did not show any increase in the brain water content compared to the control group (Table 1).

Table 1. *Changes in BCSFB, brain edema, and structural changes in rats with heat stress for 4 hours*

Parameters measured	n	Control	Heat stress 38 °C in a BOD incubator		
			1 h	2 h	4 h
BCSFB permeability#					
[131] Iodine %	5				
Whole brain		0.35 ± 0.06	0.33 ± 0.08	0.42 ± 0.08	1.88 ± 0.24**
Cisternal CSF		0.18 ≈ 0.04	0.12 ± 0.11	0.16 ± 0.12	0.76 ± 0.12**
Hippocampus		0.42 ± 0.12	0.47 ± 0.11	nd	0.84 ± 0.23**
Caudate nucleus		0.28 ± 0.08	0.32 ± 0.14	nd	0.93 ± 0.12**
Cerebellum		0.13 ± 0.08	0.16 ± 0.08	nd	0.65 ± 0.10**
Thalamus		0.48 ± 0.12	0.46 ± 0.08	nd	0.89 ± 0.14**
Hypothalamus		0.54 ± 0.21	nd	nd	0.87 ± 0.23**
Brain Stem		0.18 ± 0.08	nd	nd	0.34 ± 0.14*
Water content#	5				
Whole brain %		76.12 ± 0.18	76.04 ± 0.13	76. ± 4 ± 0.14	80.18 ± 0.24**
Hippocampus		78.43 ± 0.23	78.11 ± 0.21	78.21 ± 0.34	81.56 ± 0.34**
Caudate nucleus		77.43 ± 0.24	77.34 ± 0.32	nd	81.48 ± 0.54**
Cerebellum		74.43 ± 0.21	74.33 ± 0.32	nd	79.34 ± 0.23**
Thalamus		75.21 ± 0.22	75.12 ± 0.33	nd	78.56 ± 0.23**
Hypothalamus		74.54 ± 0.12	nd	nd	76.45 ± 0.23**
Brain Stem		68.54 ± 0.12	nd	nd	69.78 ± 0.12**
Structural changes	5				
Neuronal damage		nil	nil	nil	++++
Glial cell injury		nil	nil	±?	++++
Myelin damage		nil	nil	±?	++++

Values are Mean ± SD of 5 rats in each group.
BCSFB Blood-cerebrospinal fluid barrier; *BOD* biological oxygen demand; # tissue sample size (135–180 mg); *CSF sample* 50 to 80 µl; ±? uncertain; ++++ Severe cell damage; *nil* absent; *nd* not done; * $p < 0.05$; ** $p < 0.01$ (compared to control); ANOVA followed by Dunnett's test from 1 control.

Morphological alterations

Morphological analysis showed degeneration of choroidal epithelial cells and underlying ependyma in rats subjected to 4 hours of WBH (Fig. 1). The ventricular space appeared to be dilated and the underlying neuropil showed neurodegenerative changes. Neuronal damage, edematous expansion, and edema in hippocampus, cerebral cortex, thalamus, hypothalamus, and brain stem were very common in 4-hour heat-stressed rats (Fig. 1). On the other hand, rats subjected to 1 or 2 hours of WBH did not show structural changes in the brain or spinal cord (results not shown).

Discussion

The salient new finding of the present investigation is a marked increase in BCSFB permeability to Evans blue and radioiodine tracer following 4 hours of WBH in rats, a feature not observed in animals exposed to 1 or 2 hours of heat exposure. These observations suggest that WBH, depending on its duration, is capable of disrupting the BCSFB to large molecule tracers.

Our observations further show that leakiness of the BCSFB is associated with marked cellular changes in several brain regions located within the cerebral ventricles or adjacent regions. Thus, profound cell damage is seen in the hippocampus, caudate nucleus, thalamus, hypothalamus, cerebellum, and brain stem. This indicates that alterations in the BCSFB are somehow contributing to neurodegenerative changes in WBH.

The BCSFB resides in the choroidal epithelial cells that are connected with tight junctions [5, 6, 16, 17, 22]. It is believed that the tightness of the BCSFB is comparable to that of the blood-brain barrier (BBB) located within the cerebral capillary endothelium containing tight junctions [6, 22, 28]. Infusion of hyperosmolar solutions into the internal carotid artery is known to shrink the endothelial cells of the cerebral capillaries and widen the tight junctions leading to breakdown of the BBB [22, 34]. However, it is not known if, under identical conditions, the BCSFB is also compromised.

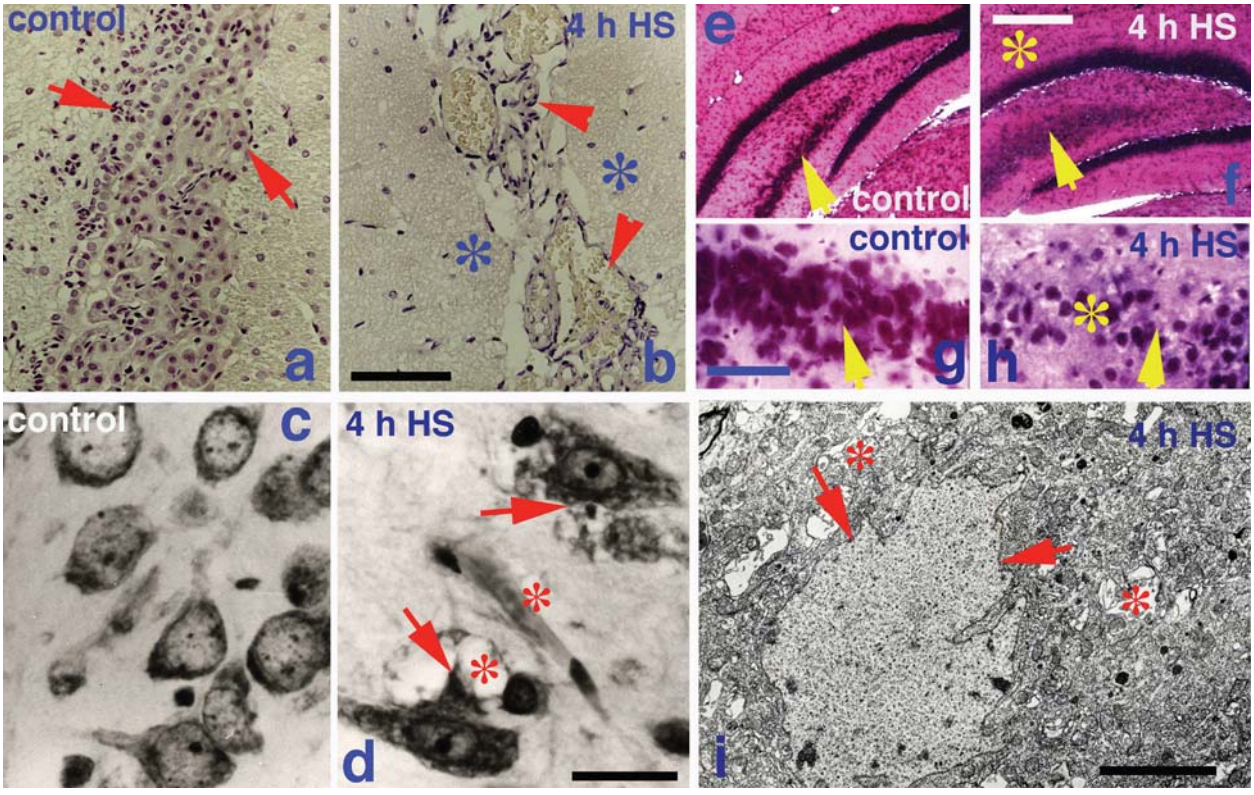

Fig. 1. Structural changes in choroidal epithelium, hippocampus, and cerebral cortex following 4 hours of whole-body hyperthermia at 38 °C. Degeneration of choroidal epithelium (arrowheads) in the lateral ventricle of a 4-hour heat-stressed rat (b) is clearly seen compared to epithelium from a normal animal (a). Damaged nerve cells (arrows) and edema (*) are evident in the cerebral cortex (d) and hippocampus CA4 (f, h) regions in heat-stressed rat compared to control (c, e, g). At the ultrastructural level, the irregular shape of one nerve cell nucleus (arrow, i) in a heat-stressed rat is apparent. Dark and condensed cytoplasm and karyoplasm is clearly visible in the cerebral cortex. Bars: a, b, e, f = 100 μm, c, d = 30 μm, g, h = 20 μm, i = 500 nm

Alterations in the composition of CSF and/or its osmolality are known to occur following lipopolysaccharide-induced fever or heat stress in rabbits [11, 12]. WBH is known to increase plasma viscosity and probably alters the plasma tonicity [2, 4, 44]. Thus, it is possible that hyperosmolality of plasma and/or CSF following WBH could be an important factor in disruption of the BCSFB.

The microvessels supplying choroid plexus are leaky [6, 22]. Thus, choroidal epithelial cells are in direct contact with the hyperosmolar blood plasma [22]. Furthermore, CSF hyperosmolality can also affect the tight junction permeability of the choroidal epithelium from the ependymal side [17]. In addition, the choroid epithelial membrane is subjected to osmotic stress in WBH that could result in increased membrane damage. The structural changes seen in the choroid epithelium and underlying ependymal area are in line with this hypothesis. To confirm these points further, ultrastructural investigations of choroidal epithelium and the tight junctions in WBH are needed.

An increase in Evans blue and radioiodine in the CSF samples obtained from rats subjected to 4 hours of WBH supports the idea of a breakdown of the BCSFB. Evans blue or radioiodine, when injected into the circulation, binds to the endogenous serum proteins [22]. In the present study, administering about 1 molecule of Evans blue binds to 12 molecules of serum albumin in vivo [22, 30]. Therefore, extravasation of Evans blue in the CSF indicates leakage of serum protein complex across the choroid plexus epithelium [30]. Extravasation of serum proteins into the CSF compartment alters the osmotic gradient across the choroid plexus epithelium and the tight junctions resulting in transport of water and other solutes from the vascular compartment leading to edema formation [22, 23, 30].

An increase in the water content of various intracerebral structures following WBH is in line with this

hypothesis. Since WBH is known to disrupt BBB in these areas as well [35, 40], a possibility exists that breakdown of the BCSFB will further aggravate regional brain edema formation due to percolation of CSF rich in serum proteins and alterations in CSF to tissue osmotic gradients. Accumulation of serum proteins in the extracellular fluid is likely to initiates a series of cellular and molecular events leading to cell injury and death [28]. The damaged nerve cells, occurrence of sponginess, vacuolation, and edema in many brain areas [28] following 4 hours of WBH are consistent with this idea.

The concept that breakdown of the BCSFB contributes to edema formation and cell injury in WBH is supported by the fact that the short duration of heat exposure (1 or 2 hours) is not associated with leakage of tracers into the CSF or an increase in water content and cell damage. These observations suggest that the magnitude and severity of WBH is primarily responsible for BCSFB damage and brain pathology.

It is unlikely that simple heating of animals following 4 hours of WBH is directly associated with BCSFB leakage [36, 37, 40]. This is evident from the findings that when anesthetized animals are subjected to 4 hours of WBH, no disruption of the BCSFB is observed [Sharma and Johanson, unpublished observation]. Thus, there is reason to believe that WBH-induced alterations in the plasma and CSF composition play an important role in BCSFB disruption. Recent findings in our laboratory suggest that CSF is a conduit of several neurohormones and is capable of transporting several hormones and growth factors in various disease processes [16]. Thus, it is possible that CSF is playing an active role in neurodegeneration and/or neuroprotection. To further explore potential therapeutic strategies involving the CSF microenvironment, the administration of neurohormones, growth factors, or growth hormone into the cerebroventricular spaces should be done in WBH, an approach currently being examined in our laboratory.

Conclusion

In conclusion, our novel observations suggest that hyperthermia induced by WBH is capable of breaking down the BCSFB and contributing to cell and tissue injury in the CNS. It would be important to see whether neuroprotective drugs in heat stress are able to attenuate BCSFB damage in WBH, a subject requiring additional investigation.

Acknowledgments

This investigation was supported by grants from Swedish Medical Research Council (2710, HSS); Astra-Zeneca, Mölndal (HSS), Sweden; National Institutes of Health (NS 27601, CEJ), USA; Alexander von Humboldt Foundation (HSS), Germany; The University Grants Commission (HSS), New Delhi, India; and The Indian Council of Medical Research (HSS), New Delhi, India. The expert technical assistance of Kärstin Flink, Kerstin Rystedt, Franziska Drum, and Katherin Kern, and secretarial assistance of Aruna Sharma are greatly appreciated.

References

1. Belmin J, Golmard JL (2005) Mortality related to the heatwave in 2003 in France: forecasted or over the top? Presse Med 34: 627–628 [in French]
2. Bouchama A (2004) The 2003 European heat wave. Intensive Care Med 30: 1–3
3. Bouchama A, Knochel JP (2002) Heat stroke. N Engl J Med 346: 1978–1988
4. Bouchama A, Roberts G, Al Mohanna F, El-Sayed R, Lach B, Chollet-Martin S, Ollivier V, Al Baradei R, Loualich A, Nakeeb S, Eldali A, de Prost D (2005) Inflammatory, hemostatic, and clinical changes in a baboon experimental model for heatstroke. J Appl Physiol 98: 697–705
5. Bouchaud C, Bouvier D (1978) Fine structure of tight junctions between rat choroidal cells after osmotic opening induced by urea and sucrose. Tissue Cell 10: 331–342
6. Bradbury MWB (1979) The concept of a blood-brain barrier. Wiley, Chichester, England
7. Centers for Disease Control and Prevention (CDC) (2005) Heat-related mortality – Arizona, 1993–2002, and United States, 1979–2002. MMWR Morb Mortal Wkly Rep 54: 628–630
8. Conti S, Meli P, Minelli G, Solimini R, Toccaceli V, Vichi M, Beltrano C, Perini L (2005) Epidemiologic study of mortality during the Summer 2003 heat wave in Italy. Environ Res 98: 390–399
9. Davis RE, Knappenberger PC, Michaels PJ, Novicoff WM (2003) Changing heat-related mortality in the United States. Environ Health Perspect 111: 1712–1718
10. Dewhirst MW, Viglianti BL, Lora-Michiels M, Hanson M, Hoopes PJ (2003) Basic principles of thermal dosimetry and thermal thresholds for tissue damage from hyperthermia. Int J Hyperthermia 19: 267–294
11. Frosini M, Sesti C, Palmi M, Valoti M, Fusi F, Mantovani P, Bianchi L, Della Corte L, Sgaragli G (2000) The possible role of taurine and GABA as endogenous cryogens in the rabbit: changes in CSF levels in heat-stress. Adv Exp Med Biol 483: 335–344
12. Frosini M, Sesti C, Palmi M, Valoti M, Fusi F, Mantovani P, Bianchi L, Della Corte L, Sgaragli G (2000) Heat-stress-induced hyperthermia alters CSF osmolality and composition in conscious rabbits. Am J Physiol Regul Integr Comp Physiol 279: R2095–R2103
13. Gauss H, Meyer KA (1917) Heat stroke: report of one hundred and fifty-eight cases from Cook County Hospital, Chicago. Am J M Sc 154: 554–564
14. Haveman J, Sminia P, Wondergem J, van der Zee J, Hulshof MC (2005) Effects of hyperthermia on the central nervous system: What was learnt from animal studies? Int J Hyperthermia 21: 473–487
15. Hildebrandt B, Hegewisch-Becker S, Kerner T, Nierhaus A,

Bakhshandeh-Bath A, Janni W, Zumschlinge R, Sommer H, Riess H, Wust P; The German Interdisciplinary Working Group on Hyperthermia (2005) Current status of radiant whole-body hyperthermia at temperatures > 41.5 degrees C and practical guidelines for the treatment of adults. The German 'Interdisciplinary Working Group on Hyperthermia'. Int J Hyperthermia 21: 169–183
16. Jhanson C, Duncan J, Baird A, Stopa E, McMillan P (2005) Choroid plexus: A key player in neuroprotection and neurodegeneration. Int J Neuroprotec Neuroregen 1: 77–85
17. Johanson CE, Foltz FM, Thompson AM (1974) The clearance of urea and sucrose from isotonic and hypertonic fluids perfused through the ventriculo-cisternal system. Exp Brain Res 20: 18–31
18. Kaiser R, Rubin CH, Henderson AK, Wolfe MI, Kieszak S, Parrott CL, Adcock M (2001) Heat-related death and mental illness during the 1999 Cincinnati heat wave. Am J Forensic Med Pathol 22: 303–307
19. Katschinski DM, Wiedemann GJ, Longo W, d'Oleire FR, Spriggs D, Robins HI (1999) Whole body hyperthermia cytokine induction: a review, and unifying hypothesis for myeloprotection in the setting of cytotoxic therapy. Cytokine Growth Factor Rev 10: 93–97
20. Malamud N, Haymaker W, Custer RP (1946) Heat stroke. A clinicopathological study of 125 fatal cases. Milit Surg 99: 397–449
21. Moore R, Mallonee S, Sabogal RI, Zanardi L, Redd J, Malone J (2002) Heat-related deaths – four states, July–August 2001, and United States, 1979–1999. JAMA 288: 950–951
22. Rapoport SI (1976) Blood-brain barrier in physiology and medicine. Raven Press, New York, pp 1–380
23. Reulen HJ, Tsuyumu M, Tack A, Fenske AR, Prioleau GR (1978) Clearance of edema fluid into cerebrospinal fluid. A mechanism for resolution of vasogenic brain edema. J Neurosurg 48: 754–764
24. Robins HI, Peterson CG, Mehta MP (2003) Combined modality treatment for central nervous system malignancies. Semin Oncol 30: 11–22
25. Scoville SL, Gardner JW, Magill AJ, Potter RN, Kark JA (2004) Nontraumatic deaths during US Armed Forces basic training, 1977–2001. Am J Prev Med 26: 205–212
26. Sharma HS (1982) Blood-brain barrier in stress [PhD Thesis] Banaras Hindu University, Varanasi, India, pp 1–85
27. Sharma HS (1999) 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 830: 1–94
28. Sharma HS (2004) Blood-brain and spinal cord barriers in stress. In: Sharma HS, Westman J (eds) Blood-spinal cord and brain barriers in health and disease. Elsevier Academic Press, San Diego, pp 231–298
29. Sharma HS (2005) Heat-related deaths are largely due to brain damage. Indian J Med Res 121: 621–623
30. Sharma HS (2005) Methods to induce brain hyperthermia. In: Costa E (ed) Current protocols in toxicology, Suppl 23. John Wiley Inc, New York, pp 1–26
31. Sharma HS (2005) Alterations of amino acid neurotransmitters in hyperthermic brain injury. J Neural Transm [in press]
32. Sharma HS, Dey PK (1986) Probable involvement of 5-hydroxytryptamine in increased permeability of blood-brain barrier under heat stress in young rats. Neuropharmacology 25: 161–167
33. Sharma HS, Dey PK (1987) 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 424: 153–162
34. Sharma HS, Cervós-Navarro J (1990) Brain oedema and cellular changes induced by acute heat stress in young rats. Acta Neurochir [Suppl] 51: 383–386
35. Sharma HS, Westman J (1998) Brain functions in hot environment. Elsevier, Amsterdam, pp 1–516
36. Sharma HS, Hoopes PJ (2003) Hyperthermia induced pathophysiology of the central nervous system. Int J Hyperthermia 19: 325–354
37. Sharma HS, Cervós-Navarro J, Dey PK (1991) 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 4: 104–124
38. Sharma HS, Cervós-Navarro J, Dey PK (1991) Acute heat exposure causes cellular alteration in cerebral cortex of young rats. Neuro Report 2: 155–158
39. Sharma HS, Westman J, Cervós-Navarro J, Nyberg F (1997) Role of neurochemicals in brain edema and cell changes following hyperthermic brain injury in the rat. Acta Neurochir [Suppl] 70: 269–274
40. Sharma HS, Westman J, Nyberg F (1998) Pathophysiology of brain edema and cell changes following hyperthermic brain injury. Prog Brain Res 115: 351–412
41. Sharma HS, Lundstedt T, Flardh M, Westman J, Post C, Skottner A (2003) Low molecular weight compounds with affinity to melanocortin receptors exert neuroprotection in spinal cord injury – an experimental study in the rat. Acta Neurochir [Suppl] 86: 399–405
42. Stott PA, Stone DA, Allen MR (2004) Human contribution to the European heatwave of 2003. Nature 432: 559–560
43. Sweeney KG (2002) Heat-related deaths. J Insur Med 34: 114–119
44. Thrall DE, Page RL, Dewhirst MW, Meyer RE, Hoopes PJ, Kornegay JN (1986) Temperature measurements in normal and tumor tissue of dogs undergoing whole body hyperthermia. Cancer Res 46: 6229–6235
45. Wrba E, Nehring V, Chang RC, Baethmann A, Reulen HJ, Uhl E (1997) Quantitative analysis of brain edema resolution into the cerebral ventricles and subarachnoid space. Acta Neurochir [Suppl] 70: 288–290

Correspondence: Hari Shanker Sharma, Department of Surgical Sciences, Anesthesiology & Intensive Care Medicine, University Hospital, Uppsala, Sweden. e-mail: Sharma@surgsci.uu.se