Introduction

Heatstroke is defined as a form of excessive hyperthermia (>40 °C) associated with a systemic inflammatory response that leads to multi-organ dysfunction in which central nervous system (CNS) disorders such as delusion, convulsion and coma predominate [1]. Our recent results have demonstrated that heatstroke rodents display hypotension, systemic inflammatory responses, hypothalamic ischemia and neuronal damage, and multi-organ dysfunction [2–4].

Kynurenic acid (KYNA) or its metabolic precursor L-kynurenine may be of therapeutic value in neurodegenerative diseased models [5–8]. For example, systemically administered high doses of KYNA had a neuroprotective effect in the gerbil model of global ischemia [7]. Both the homocysteine-induced impairment of endothelial cells [8] and the motility and inflammatory activation in the early phase of acute experimental colitis in the rat [9] were significantly reduced by administration of KYNA.

The aim of this study was to investigate whether the heatstroke induced hypotension, systemic inflammatory responses, hypothalamic ischemia and damage, and multi-organ dysfunction could be attenuated by KYNA preconditioning. Accordingly, the temporal profiles of the apoptotic cell numbers of spleen, kidney, liver, lung, and hypothalamus,
the serum levels of systemic inflammatory response molecules (e.g., tumor necrosis factor-α [TNF-α], intracellular adhesion molecule-1 [ICAM-1], and interleukin-10 [IL-10]), body core temperature (Tco), and mean arterial pressure (MAP) during heatstroke were assessed in rats treated with or without KYNA (3–30 mg/kg of body weight, intravenously, 4 h before the start of thermal experiments).

**Materials and methods**

**Animals**

Adult male Sprague-Dawley rats (weight 263±15 g) were obtained from the Animal Resource Center of the National Science Council of China (Taipei, Taiwan). The animals were housed 4 in a group at an ambient temperature of 22±1 °C, with a 12-h light/dark cycle. Pellet rat chow and tap water were available ad libitum. All protocols were approved by the Animal Ethics Committee of the Chi Mei Medical Center (Tainan, Taiwan, China) in accordance with the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health, as well as the guidelines of the Animal Welfare Act. Adequate anesthesia was maintained to abolish the corneal reflex and pain reflexes induced by tail pinching throughout all experiments (approximately 6 h) by a single intraperitoneal dose of urethane (1.4 g/kg body weight). At the end of the experiments, control rats that had survived heatstroke were killed with an overdose of urethane.

**Surgery and physiological parameter monitoring**

The right femoral artery and vein of rats were cannulated with polyethylene tubing (PE50), under urethane anesthesia, for blood pressure monitoring and drug administration. The Tco was monitored continuously by a thermocouple, while MAP and heart rate (HR) were monitored continuously with a pressure transducer.

**Induction of heatstroke**

The Tco of the anesthetized animals were maintained at about 37 °C with an infrared light lamp, except during the heat stress experiments. Heatstroke was induced by placing the animals in a folded heating pad maintained at 43 °C by circulating hot water. Survival time values (interval between the start of heat stress and animal death) were determined.

**Experimental groups**

Animals were assigned randomly to one of three groups. One group of rats, treated with an intravenous dose of vehicle solution (0.5 mL normal saline per rat) was exposed to 26 °C for up to 480 min (or to the end of the experiments). This group of rats was used as normothermic controls. The second group was treated with the same dose of vehicle solution 4 h before the initiation of heat exposure (43 °C for 68 min) and was used as vehicle-treated heatstroke animals. The third group of rats was intravenously treated with KYNA (30–100 mg/kg of body weight in 0.3 mL saline; Sigma Chemical Co, St Louis, MO, USA) 4 h before the start of thermal experiments. The last two groups of rats were exposed to heat stress (43 °C) for exactly 68 min to induce heatstroke and were then allowed to recover at 26 °C. Physiological parameters and survival time were observed for up to 480 min (or to the end of the experiments).

In Experiment 1, the survival time values for normothermic rats, vehicle-treated heatstroke rats, and KYNA-treated heatstroke rats were determined randomly.

In Experiment 2, values of both Tco and MAP for normothermic rats (NT), vehicle-treated heatstroke rats (VEH+HS), and KYNA-treated heatstroke rats (KYNA+HS) were determined at 0 min, 68 min, or 85 min after the initiation of heat exposure in heatstroke rats or the equivalent time in normothermic controls.

**Neuronal damage score**

Eighty-five minutes after the start of heat stress, animals were killed by an overdose of urethane, and the brains were fixed in 10% neutral-buffered formalin for at least 24 h. The brain was removed and embedded in paraffin blocks. Serial sections (10 μm thick) through the hypothalamus were stained with hematoxylin and eosin for microscopic evaluation. The extent of neuronal damage was scored on a scale of 0–3, modified from the grading system of Pulsinelli et al[10], in which 0 is normal, 1 indicates approximately 30% of the neurons are damaged, 2 indicates that approximately 60% of the neurons are damaged, and 3 indicates that 100% of the neurons are damaged. Each hemisphere was evaluated independently by an examiner blinded to the experimental conditions.

**The terminal deoxynucleotidyl transferase-mediated and dUTP-biotin nickened-labeling, TUNEL, staining**

The TUNEL assay was performed using the apoptotic cells in different tissue samples including the spleen, kidney, liver, lung and hypothalamus. Color was developed using 3,3′-diaminobenzidine tetrachloride (Sigma chemical Co, St Louis, MO, USA). Sections were treated with xylene and ethanol to remove paraffin and for dehydration. They were then washed with phosphate buffered saline (PBS) and incubated in 3% hydrogen peroxide solution for 20 min. The sections were treated with 5 μg/mL proteinase k for 2 min at room temperature, and rewash in PBS (0.1 mol/L, pH 7.4). The sections were then treated with a TUNEL reaction mixture (terminal deoxynucleotidyl transferase nucleotide mixture, Roche, Mannheim, Germany) at 37 °C for 1 h, and the sections were washed with distilled water. They were then incubated in anti-fluorescein antibody conjugated with horseradish peroxidase at room temperature for 30 min, washed and visualized using the avadinbiotin complex technique and 0.05% 3,3′-diaminobenzidine tetrachloride as a chromogen. The numbers of TUNEL-positive cells were counted by a patholo-
gist at 200× magnification, 30 fields per section. Blinding was performed for the pathologist’s grading of results.

**Measurement of serum TNF-α, ICAM-1, and IL-10 levels**

Eighty-five minutes after the start of heat stress, blood samples were collected, immediately separated, and stored at -80 °C until they could be assayed. We used commercially available ELISA kits for the determination of serum TNF-α, ICAM-1, and IL-10 levels (Quantikine, R&D Systems Inc, Minneapolis, MN, USA) according to the manufacturer’s instructions.

**Statistical analysis**

All data are expressed as the mean±standard deviation. One-way analysis of variance with Tukey’s multiple comparisons test was used for serum markers, Tco, MAP, survival time, and apoptotic cell numbers. The Wilcoxon test was used for histological assessment. Significant differences were established at *P* < 0.05. For all statistical analyses, SPSS software version 10.0 (SPSS Inc, Chicago, IL, USA) was used.

**Results**

**KYNA prolonged survival time values during heatstroke**

As depicted in Figure 1, body Tco and MAP increased to values of about 41 °C and about 140 mmHg, respectively at 68 min after the start of heat exposure (43 °C). However, 85 min after the start of heat stress, the Tco and MAP reached new values of about 41 °C and about 40 mmHg, respectively. The instant (85 min after heat exposure) in which Tco rose above 41 °C and MAP dropped about 40 mmHg was arbitrarily defined as the time point for the onset of heatstroke[^3,11,12]. At 68 min, the heating pad was removed, and the animals were allowed to recover at room temperature (26 °C). The survival time values during heatstroke for vehicle-treated rats were decreased from the control values of 475–485 min (*n*=6) to new values of 83–95 min (*n*=6). Treatment with KYNA (30–100 mg per kg of body weight, intravenously) 4 h before the start of heat stress significantly (*P*<0.05) and dose-dependently decreased the survival time to new values of 152–356 min compared with normothermic rats (Table 1).

**Table 1.** The survival time values for normothermic rats, vehicle-treated rats, and KYNA-treated rats. Data were mean±SD. *n*=6. *P*<0.05, in comparison with group 1; *P*<0.05, in comparison with group 2.

| Treatment groups                        | Survival time (min) |
|-----------------------------------------|---------------------|
| Normothermic rats                       | 480±5               |
| Vehicle-treated heatstroke rats         | 89±6*               |
| KYNA (10 mg/kg, iv)-treated heatstroke rats | 152±7*             |
| KYNA (30 mg/kg, iv)-treated heatstroke rats | 191±9*             |
| KYNA (100 mg/kg, iv)-treated heatstroke rats | 356±17*            |

All heatstroke rats which had heat exposure (43 °C) were withdrawn exactly at 68 min and then allowed to recover at room temperature (26 °C). Heatstroke rats were killed 85 min after the start of heat stress, whereas the normothermic rats were killed about 480 min after the start of experiment (or at the experiment end). Vehicle or KYNA was injected 4 h before the start of experiments.

**KYNA protected against hypotension but not hyperthermia during heatstroke**

As shown in this Figure 1, heat stress induced significant (*P*<0.05) increase in both Tco and MAP in vehicle-treated heatstroke rats at 68 min. However, 17 min after termination of heat stress (or 85 min after the initiation of heat exposure), the values of MAP but not Tco were significantly (*P*<0.05) lower than those of the normothermic rats (40 mmHg) vs 98 mmHg; Figure 1). The heat-induced hypotension, but not hyperthermia, was significantly (*P*<0.05) reduced by KYNA preconditioning.

**KYNA attenuated hypothalamic neuronal degeneration and apoptosis during heatstroke**

As shown in Table 2, after the onset of heatstroke, the hypothalamic neuronal damage scores were higher in animals treated vehicle compared with the normothermic controls. Histopathologic verification revealed that heatstroke caused cell body shrinkage, pyknosis of the nucleus, loss of Nissl substance, and disappearance of the nucleus in the hypothalamus of vehicle-treated rats (Figure 2). The figure also showed that the heat-induced hypothalamic neuronal degeneration were greatly reduced in KYNA-treated heatstroke rats (*P*<0.05).

Figure 3 showed the effects of heat exposure on the number of TUNEL-positive cells in the hypothalamus of normothermic
controls, vehicle-treated heatstroke rats, and KYNA-treated heatstroke rats. After the onset of heatstroke (or 85 min after the start of heat stress), the number of TUNEL-positive cells of the hypothalamus was greater ($P<0.05$) in vehicle-treated heatstroke rats than in the normothermic controls. However, increase of TUNEL-positive cells in the hypothalamus of heatstroke rats was greatly attenuated by KYNA. A typical example of TUNEL staining of the hypothalamus was shown in the top panel of Figure 3.

**KYNA attenuated spleen, kidney, liver, and lung apoptosis during heatstroke**

Figures 4–7 summarized the effects of heat exposure on the number of TUNEL-positive cells in the spleen, the kidney, the liver, and the lung, respectively, of normothermic controls, vehicle-treated heatstroke rats, and KYNA-treated heatstroke rats. After the onset of heatstroke, the number of TUNEL-positive cells of these organs were greater ($P<0.05$) in vehicle-treated heatstroke rats than in the normothermic controls. However, increase of TUNEL-positive cells in these organs of heatstroke rats was greatly ($P<0.05$) attenuated by KYNA pre-conditioning. The typical examples of TUNEL stainings of these organs were shown in the top panel of Figures 4–7, respectively.

**KYNA up-regulated serum IL-10 levels but down-regulated serum TNF-α and ICAM-1 levels during heatstroke**

Figure 8 showed the serum levels of ICAM-1, TNF-α, and IL-10 among the three experimental groups. Compared with the normothermic controls, vehicle-treated heatstroke rats had higher levels ($P<0.05$) of ICAM-1 and TNF-α after the onset of heatstroke. The increase in the serum levels of these two markers caused by heatstroke were significantly reduced by KYNA pre-conditioning. However, compared with the vehicle-treated rats, KYNA-treated rats had higher ($P<0.05$) serum levels of IL-10 after the onset of heatstroke.
Discussion

A hypothesis of how heat stress leads to multi-organ dysfunction has been proposed\cite{12,13}. Heat stress stimulates metabolism and progressively reduces blood flow to critical splanchnic and brain tissues. Increased metabolic demand coupled with reduced splanchnic and brain blood flow generates cellular hypoxia, compromises cellular energy production, and produces derangements in intracellular Ca\textsuperscript{2+} homeostasis. As heat stress continues, hypoxic cells can produce multiorgan dysfunction and inflammation. Although the severity of heatstroke depends on the degree of hyperthermia and its duration\cite{14}, normal volunteers can passively endure a core temperature of about 42 °C with none or minimal tissue injury\cite{15,16}. Indeed, as demonstrated in the present results, KYNA treatment significantly prevented the occurrence of heat-induced multi-organ damage and inflammation without
KYNA is a kynurenine pathway metabolite that affects the induced hyperthermia. It should be mentioned that, in the present study, all these heatstroke animals were under the general anesthesia of urethane. Although the survival time values of these heatstroke animals were prolonged by KYNA preconditioning, but all the animals died. To determine whether there was durable improvement in survival, anesthetized and unrestrained animals with or without KYNA should be exposed to heat stress in future studies.

Recent findings have documented that unanesthetized, unrestrained rodents display thermoregulatory deficits (e.g., heatstroke animals showed hypothermia when exposed to room temperature, 26 °C) 4 h after the initiation of heat stress[17, 18]. The heatstroke-induced thermoregulatory deficits may have resulted from neuronal apoptosis and cell degeneration in the hypothalamus (as demonstrated in the current study). The current results further showed that the hypothalamic apoptosis and neuronal degeneration that occurred during heatstroke in rats could be significantly ameliorated by KYNA treatment.

KYNA is a tryptophan metabolite formed as part of the kynurenine pathway. It has been shown that KYNA is an antagonist of the glycine site of NMDA and of the α-7 nicotinic acetylcholine receptors as well as a ligand for the orphan G-protein coupled receptor, GPR35. Altered blood-brain-barrier (BBB) permeability and brain injury occurred during heatstroke in rats[29]. In addition, human umbilical cord derived CD34+ cells were found to be able to pass into the hyperthermic brain to attenuate heat-induced brain injury and damage in rats[30]. It could be derived from the foregoing statements that systemic delivery of KYNA might ameliorate multiorgan dysfunction that occurred during heatstroke via both the central and peripheral components of inhibition. In particular, KYNA might have achieved its central action via passing through the disrupted blood-brain-barrier.

It is generally believed that activation of the excitatory amino acid receptors plays an important role in neuronal death in stroke[31], as well as in the grey matter ischemia[32]. Kynurenic acid is one of the few known endogenous NMDA receptor inhibitors[33]. Our data and theoretical consideration suggest that KYNA or its metabolic precursor L-kynurenine may be of therapeutic value in heatstroke syndromes. However, the use of KYNA as a neuroprotective agent can be excluded because it is barely able to cross the BBB, whereas L-kynurenine is transported much more readily across the BBB[34].

In additional to inducing neuronal damage to brain tissues, heatstroke caused severe damage to the lung, kidney, liver, and spleen[22-24]. The current results further demonstrated that heat-induced multi-organ damage could be significantly ameliorated by KYNA preconditioning in the rat. In fact, the glutamate receptors are also present in pancreas, gut, kidney, liver, lung, spleen, and testis[35, 36]. Experiments performed on bovine aorta endothelial cell cultures showed that KYNA exerted a protective activity against the homocysteine-induced impairment of endothelial cells[8]. The addition of KYNA significantly increased endothelial cell migration and proliferation, which was diminished by homocysteine. KYNA also protected cells against homocysteine-induced cytotoxicity. KYNA was also shown to decrease motility and inflammatory activation in the early phase of acute experimental colitis in the rat[9].

A high affinity glutamate/aspartate transport system exists in pancreatic islets of langerhans and that this system contributes to a glutamatergic signaling pathway that can modulate...
glucose-inducible insulin secretion[35]. Our previous results[37] showed that, after the onset of heatstroke in the rat, hypotension accompanied by no change in blood levels of glucose was observed.

It has been documented that the pathophysiological responses exerted during heatstroke are the results of a systemic inflammatory response that ensues following thermal injury rather than a direct effect of hyperthermia[1]. The serum TNF-α and ICAM-1 levels can be regarded as markers for the systemic inflammatory response as they indirectly reflect the whole body production of both TNF-α and ICAM-1 in various organs[38–41]. The expression of adhesion molecules can be induced by TNF-α[38]. The adhesion molecule ICAM-1 mediates firm adhesion between leukocytes and endothelial cells and contributes to the migration of leukocytes from post-capillary venues into the reperfused tissue[42, 43]. ICAM-1 initiates adhesion and transendothelial migration of circulating leukocytes[42]. Evidence has also suggested that IL-10 may have a therapeutic potential in acute and chronic inflammatory disease[44]. Exogenous administration of recombinant IL-10 protects mice from lethal endotoxemia by reducing TNF-α release[44]. In the present study, we showed that KYNA preconditioning increased the serum levels of IL-10 and decreased the serum levels of both TNF-α and ICAM-1, and prolonged the survival time during heatstroke. These observations suggested that KYNA might improve survival of heatstroke rats via reducing systemic inflammatory response in the rat.

In summary, the present data indicated that KYNA might exert a protective role on multiple organs during heatstroke through the following mechanisms: (A) Anti-inflammation: KYNA inhibited the up-regulation of systemic inflammatory response molecules such as TNF-α and ICAM-1 but enhanced the up-regulation of IL-10; (B) Anti-hypotension: KYNA reduced heat-induced splanchic and brain ischemia; (C) Anti-apoptosis: KYNA inhibited heat-induced hypothalamic neuronal apoptosis and degeneration and multi-organ dysfunction.

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Author contribution

Yi-chang HSIEH and Sheng-hsien CHEN designed research; Yi-chang HSIEH and Ruei-feng CHEN performed research; Yi-shian YEH contributed new analytical tools and reagents; Sheng-hsien CHEN and Ju-hsiang HSIEH analyzed data; Mao-tsun LIN and Sheng-hsien CHEN wrote the paper.

References

1. Bouchama A, Knochel JP. Heat stroke. N Engl J Med 2002; 346: 1978–88.
2. Chang CK, Chang CP, Chiu WT, Lin MT. Prevention and repair of circulatory shock and cerebral ischemia/injury by various agents in experimental heatstroke. Curr Med Chem 2006; 13: 3145–54.
3. Liu WS, Chen CT, Foo NH, Huang HR, Wang JJ, Chen SH, et al. Human umbilical cord blood cells protect against hypothalamic apoptosis and systemic inflammation response during heatstroke in rats. Pediatr Neonatol 2009; 50: 208–16.
4. Yang HH, Chang CP, Cheng JT, Lin MT. Inhibition of acute lung inflammation and injury is a target of brain cooling after heatstroke injury. J Trauma 2010; in press.
5. Nemeth H, Robotka T, Toldi J, Vecsei L. Kynurenic acids in the central nervous system: recent developments. Centr Nerv Sys Agents Med Chem 2007; 7: 45–56.
6. Salvati P, Utkar M, Dho L, Rosa B, Cini M, Marconi M, et al. Brain concentrations of kynurenic acid after a systemic neuroprotective dose in the gerbil model of global ischemia. Prog Neuropsychopharmacol Biol Psychiatry 1999; 23: 741–52.
7. Wejksza K, Rzeski W, Turski WA. Kynurenic acid protects against the homocysteine-induced impairment of endothelial cells. Pharmacol Rep 2009; 61: 751–6.
8. Varga G, Erces D, Fazekas B, Fulop M, Kovacs T, Kaszaki J, et al. N-Methyl-D-aspartate receptor antagonism decreases motility and inflammatory activation in the early phase of acute experimental colitis in the rat. Neurogastroenterol Motil 2010; 22: 217–25.
9. Pulsinelli WA, Levy DE, Duffy TE. Regional cerebral blood flow and glucose metabolism following transient forebrain ischemia. Ann Neurol 1982; 11: 499–509.
10. Chen SH, Chang FM, Tsai YC, Huang KF, Lin MT. Resuscitation from experimental heatstroke by transplantation of human umbilical cord blood cells. Crit Care Med 2005; 33: 1377–83.
11. Chen SH, Chang FM, Tsai YC, Huang KF, Lin CL, Lin MT. Infusion of human umbilical cord blood cells protect against cerebral ischemia and damage during heatstroke in the rat. Exp Neurol 2006; 199: 67–76.
12. Hall DM, Buettner GR, Oberley LW, Xu L, Matthes RD, Gisolfi CV. Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. Am J Physiol Heart Circ Physiol 2001; 280: H509–21.
13. Dematte JE, O’Marra K, Buescher J, Whitney CG, Forsythe S, McNamee T, et al. Near-fatal heat stroke during the 1995 heat wave in Chicago. Ann Intern Med 1998; 129: 173–81.
14. Bynum GD, Pandolf KB, Schuetze WH, Goldman RF, Lees DE, Whang-Peng J, et al. Induced hyperthermia in sedated humans and the concept of critical thermal maximum. Am J Physiol 2007; 285: R228–36.
15. Pettigrew RT, Galt JM, Ludgate CM, Horn DB, Smith AN. Circulatory and biochemical effects of whole body hyperthermia. Br J Surg 1974; 61: 727–30.
16. Chatteryee S, Premachandran S, Bagewadikar RS, Bhattacharya S, Chattopadhyay S, Poddub TL. Arginine metabolic pathways determine its therapeutic benefit in experimental heatstroke: role of Th1/Th2 cytokine balance. Nitric Oxide 2006; 15: 408–16.
17. Shen KH, Lin CH, Chang HK, Chen WC, Chen SH. Premarin can act via estrogen receptors to rescue mice from heatstroke-induced lethality. Shock 2008; 30: 668–74.
18. Birch PJ, Grossman CJ, Hayes AG. Kynurenic acid antagonises responses to NMDA via an action at the strychnine-insensitive glycine receptor. Eur J Pharmacol 1988; 154: 85–7.
19. Hilmas C, Pereira EF, Alkondon M, Rassoulpour A, Schwarcz R, Albuquerque EX. The brain metabolite kynurenic acid inhibits alpha7 nicotinic receptor activity and increases non-alpha7 nicotinic receptor expression: physiopathological implications. J Neurosci 2001; 21: 7463–73.
20 Wang J, Simonavicius N, Wu X, Swaminath G, Reagan J, Tian H, et al. Kynurenic acid as a ligand for orphan G protein-coupled receptor GPR35. J Biol Chem 2006; 281: 22021–8.

21 Hou ST, MacManus JP. Molecular mechanisms of cerebral ischemia-induced neuronal death. Int Rev Cytol 2002; 221: 93–148.

22 Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science 1993; 262: 689–95.

23 Prass K, Dirnagl U. Glutamate antagonists in therapy of stroke. Restor Neurol Neurosci 1998; 13: 3–10.

24 Pin JP, Duvoisin R. The metabotropic glutamate receptors: structure and functions. Neuropharmacology 1995; 34: 1–26.

25 Simon RP, Young RS, Stout S, Cheng J. Inhibition of excitatory neurotransmission with kynurenate reduces brain edema in neonatal anoxia. Neurosci Lett 1986; 71: 361–4.

26 Andine P, Lehmann A, Ellren K, Wennberg E, Kjellmer I, Nielsen T, et al. The excitatory amino acid antagonist kynurenic acid administered after hypoxic-ischemia in neonatal rats offers neuroprotection. Neurosci Lett 1988; 90: 208–12.

27 Germano IM, Pitts LH, Meldrum BS, Bartkowski HM, Simon RP. Kynurenic acid inhibition of cell excitation decreases stroke size and deficits. Ann Neurol 1987; 22: 730–4.

28 Sharma HS, Westman J, Cervos-Navarro J, Nyberg F. Role of neurochemicals in brain edema and cell changes following hyperthermic brain injury in the rat. Acta Neurochir Suppl 1997; 70: 269–74.

29 Chen SH, Chang FM, Chang HK, Chen WC, Huang KF, Lin MT. Human umbilical cord blood-derived CD34⁺ cells cause attenuation of multiforgan dysfunction during experimental heatstroke. Shock 2007; 27: 663–71.

30 Choi DW. Glutamate neurotoxicity and diseases of the nervous system. Neuron 1988; 1: 623–4.

31 Dohmen C, Kumura E, Rosner G, Heiss WD, Graf R. Extracellular correlates of glutamate toxicity in short-term cerebral ischemia and reperfusion: a direct in vivo comparison between white and gray matter. Brain Res 2005; 1037: 43–51.

32 Swartz KJ, During MJ, Freese A, Beal MF. Cerebral synthesis and release of kynurenic acid: an endogenous antagonist of excitatory amino acid receptors. J Neurosci 1990; 10: 2965–73.

33 Fukui S, Schwarzw R, Rapoport SI, Takada Y, Smith QR. Blood-brain barrier transport of kynurenes: implications for brain synthesis and metabolism. J Neurochem 1991; 56: 2007–17.

34 Weaver CD, Gundersen V, Verdoorn TA. A high affinity glutamate/aspartate transport system in pancreatic islets of Langerhans modulates glucose-stimulated insulin secretion. J Biol Chem 1998; 273: 1647–53.

35 Gill SS, Mueller RW, McGuire PF, Pulido OM. Potential target sites in peripheral tissues for excitatory neurotransmission and excitotoxicity. Toxicol Pathol 2000; 28: 277–84.

36 Chou YT, Lai ST, Lee CC, Lin MT. Hypothermia attenuates circulatory shock and cerebral ischemia in experimental heatstroke. Shock 2003; 19: 388–93.

37 Wyble CW, Desai TR, Clark ET, Hynes KL, Gewertz BL. Physiologic concentrations of TNFalpha and IL-1beta released from reperfused human intestine upregulate E-selectin and ICAM-1. J Surg Res 1996; 63: 333–8.

38 Chen LW, Egan L, Li ZW, Greten FR, Kagnoff MF, Karin M. The two faces of IKK and NF-kappaB inhibition: prevention of systemic inflammation but increased local injury following intestinal ischemia-reperfusion. Nat Med 2003; 9: 575–81.

39 Kuzu MA, Koksoy C, Kuzu I, Gurhan I, Ergun H, Demirpence E. Role of integrins and intracellular adhesion molecule-1 in lung injury after intestinal ischemia-reperfusion. Am J Surg 2002; 183: 70–4.

40 Olanders K, Sun Z, Borjesson A, Dib M, Andersson E, Lasson A, et al. The effect of intestinal ischemia and reperfusion injury on ICAM-1 expression, endothelial barrier function, neutrophil tissue influx, and protease inhibitor levels in rats. Shock 2002; 18: 86–92.

41 Menger MD, Vollmar B. Adhesion molecules as determinants of disease: from molecular biology to surgical research. Br J Surg 1996; 83: 588–601.

42 Hogg N, Bates PA, Harvey J. Structure and function of intercellular adhesion molecule-1. Chem Immunol 1991; 50: 98–115.

43 Berg DJ, Kuhn R, Rajewsky K, Muller W, Menon S, Davidson N, et al. Interleukin-10 is a central regulator of the response in LPs in murine models of endotoxic shock and the Shwartzman reaction but not endotoxin tolerance. J Clin Invest 1995; 96: 2339–47.

44 Gerard C, Bruyns C, Marchant A, Abramowicz D, Vandenameele P, Delvaux A, et al. Interleukin 10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. J Exp Med 1993; 177: 547–50.