BRAIN COOLING CAUSES ATTENUATION OF CEREBRAL OXIDATIVE STRESS, SYSTEMIC INFLAMMATION, ACTIVATED COAGULATION, AND TISSUE ISCHEMIA/INJURY DURING HEAT STROKE

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ABSTRACT—The purpose of present study was to assess the therapeutic effect of hypothermic retrograde jugular vein flush (HRJVF) on heat stroke. HRJVF was accomplished by infusion of 4°C normal saline via the external jugular vein (1.7 ml per 100 g of body weight over 5 min). Anesthetized rats, immediately after the onset of heat stroke, were divided into two major groups and given the following: 36°C or 4°C normal saline intravenously. They were exposed to ambient temperature of 43°C to induce heat stroke. Another group of rats was exposed to room temperature (24°C) and used as normothermic controls. When the 36°C saline-treated rats underwent heat exposure, their survival time values were found to be 23-28 min. Resuscitation with intravenous dose of 4°C saline, immediately after the onset of heat stroke, significantly improved survival during heat stroke (208-252 min). All heat-stressed animals displayed systemic inflammation and activated coagulation, evidenced by increased tumor necrosis factor-α, prothrombin time, activated partial thromboplastin time, and D-dimer, and decreased platelet count and protein C. Biochemical markers evidenced cellular ischemia and injury/dysfunction: plasma levels of blood urea nitrogen, creatinine, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and alkaline phosphatase, and striatal levels of glycerol, glutamate, and lactate/pyruvate, dihydroxy benzoic acid, lipid peroxidation, oxidized-form glutathione reduced-form glutathione, dopamine, and serotonin were all elevated during heat stroke. Core and brain temperatures and intracranial pressure were also increased during heat stroke. In contrast, the values of mean arterial pressure, cerebral perfusion pressure, and striatal levels of local blood flow, partial pressure of O₂, total superoxide dismutase, catalase, glutathione peroxidase,
and glutathions reductase activities were all significantly lower during heat stroke. The circulatory dysfunction, systemic inflammation, hypercoagulable state, and cerebral oxidative stress, ischemia and damage during heat stroke were all significantly suppressed by HRJVF. These findings demonstrate that brain cooling caused by HRJVF therapy may resuscitate heat stroke victims by attenuating cerebral oxidative stress, systemic inflammation, activated coagulation, and tissue ischemia/injury during heatstroke.

KEYWORDS—hyperthermia, heatstroke, oxidative stress, ischemia, neuronal damage, hypotension, inflammation, coagulation
INTRODUCTION

Heatstroke is a life-threatening syndrome characterized by multiple organ dysfunction (including arterial hypotension), hyperthermia, and central nervous system disorders (1, 2). Excessive activation of systemic inflammation and hypercoagulable state may contribute to multiple organ failure and dysfunction in heat stroke (3). Hypothermia is the current therapy of choice for heatstroke because no pharmacologic agent is available (1, 2). Recently, we used the hypothermic retrograde jugular vein flush (HRVJF) to cool the brain and provided better brain protection than peripheral cold saline perfusion during heatstroke in the rat (4, 5). We concluded that HRVJF protected the brain by maintaining cerebral blood flow in rats after heatstroke. However, the true mechanism of protection exerted by brain cooling during heat stroke remains unclear.

The conventional explanation of the neuroprotective hypothermia holds that the hypothermic decline of the cerebral metabolic rate of oxygen maintains the aerobic metabolism (6, 7). However, evidence has accumulated to indicate that hypothermia may reduce tissue damage by suppressing oxidative stress (8-14). It is likely that HRVJF resuscitates heat stroke victims by attenuating cerebral oxidative stress (15), systemic inflammation, activated coagulation, and tissue ischemia/injury (3).

To test this hypothesis, the present study was undertaken in anesthetized rats to determine the effect of HRVJF on the malondialdehyde, reduced-form glutathione (GSH), oxidized-form GSH (GSSG), GSSG/GSH ratio, glutathione peroxidase (GPx) activity, glutathione reductase (GR) activity, catalase activity, and superoxide dismutase (SOD) activity in different brain structures of heatstroke rats with or without HRVJF. In addition, this study was to compare the temporal profiles of cardiovascular dysfunction, hypercoagulable state, and tissue ischemia and damage.
during heat stroke in the rat with or without HRVJF.
MATERIALS AND METHODS

Experimental animals

Experiments were performed in male adult Spraque-Dawley rats (weighing 247-348 g), which were obtained from the Animal Resource Center, National Science Council (Taipei, Taiwan, Republic of China). The animals were housed four to a cage at an ambient temperature of 22 ± 1°C, with a 12-h light / dark cycle. Pelleted rat chow and tap water were available ad libitum. The experimental protocol was approved by the Animal Committee of the Chi-Mei Foundation Hospital. Animal care and experiments were conducted according to the National Science Council guidelines. They were allowed to become acclimated for ≧1 wk. Adequate anesthesia was maintained to abolish the corneal reflex and pain reflexes induced by tail-pinching throughout all experiments by an intraperitoneal dose of sodium pentobarbital (60 mg/kg body weight). At the end of the experiments, control rats and any rats that had survived heat stroke were killed with an overdose of sodium pentobarbital.

Induction of heat stroke

Rats under anesthesia were randomized into three major groups as described in Figure 1: a) normothermic controls (NC, n=8); b) heatstroke rats treated with 36°C-saline (1.7 mL per 100 g of body weight) via the external jugular vein (cranial direction) (HS, n=8); and c) heatstroke rats treated with 4°C-saline or 24°C-saline (1.7 mL per 100 g of body weight) via the external jugular vein (cranial direction) (HS+J, n=8 for each group).

Before induction of heat stroke, the core temperature (Tco) of sodium pentobarbital-anesthetized rats was maintained at about 36°C with a folded heating pad, except during heat stress at a room temperature of 24°C. Heatstroke was induced by increasing the temperature of the folded heating pad to 43°C with circulating hot
water. The moment at which the mean arterial pressure (MAP) dropped to 25 mmHg from the peak level was taken as the onset of heat stroke (16-18). Immediately after the onset of heat stroke, the heating pad was removed and the animals were allowed to recover at room temperature (24°C). Our pilot study showed that the latency for the onset of heat stroke (interval between the start of heat exposure and the onset of heat stroke) was found to be $70 \pm 3$ min for the HS group (n=8). Physiologic parameters and survival times (intervals between the initiation of heat exposure and animal death) were then observed to 480 min (or the end of experiments). For comparison with the HS group, all HS+J group animals were exposed to heat for exactly 70 min and were then allowed to recover at room temperature (24°C).

**Surgery and physiologic parameter monitoring**

The right femoral artery and vein of rats were cannulated with polyethylene tubing (PE 50) under sodium pentobarbital anesthesia for blood pressure monitoring and drug administration. For measurement of intracranial pressure (ICP), the animals were positioned in a stereotaxic apparatus (Kopf 1406; Grass Instrument, Quincy, MA) to insert probes for Statham P23AC transducer via 20-gauge stainless-steel needled probe (diameter 0.90 mm; length 38 mm), which was introduced into the right cerebral ventricle according to the stereotaxic coordinates of Paxinos and Watson (19): A, interaural, 7.7 mm; L 2.0 mm from the midline; and H, 3.5 mm from the top of the skull. All recordings were made on a four-channel Gould polygraph. Core temperature (Tco) was monitored continuously by a thermocouple, and mean arterial pressure (MAP) and heart rate (HR) were continuously monitored with a pressure transducer. Different groups of animals were used for different sets of experiments: a) measurement of latency for onset of heat stroke (n=8); b) measurement of survival time (interval between onset of heat stroke and animal death) (n=24); c) measurement
of MAP, cerebral perfusion pressure (CPP=MAP-ICP), ICP, cerebral blood flow (CBF), brain PO$_2$, and brain temperature (Tbr) (n=24); d) measurement of Tco, MAP, HR, and striatal levels of dihydroxybenzoic acid (DHBA), glutamate, glycerol, lactate/pyruvate, dopamine, and serotonin (n=24); and measurement of levels of malondialdehyde (MDA), glutathione reductase (GR), glutathione peroxidase (GPx), reduced-form glutathione (GSH), oxidized–form glutathione (GSSG), GSSG/GSH ratio, catalase, and superoxide dismutase (SOD) in different brain structures including the hypothalamus, the cortex, the hippocampus, the striatum, the cerebellum, and the brain stem.

**Biochemical measurements**

Blood samples at 0, 70, and 85 min after initiation of heat stress were drawn by arterial femoral cannulation. Properly calibrated and controlled automated devices were used to determine complete blood counts (CellDyn 400; Abbott Diagnostics, Santa Clara, CA), liver profiles (Hitachi 912; Mannheim-Boehringer, Mannheim, Germany), and coagulation profiles (BCS; Dade Behring, Miami, FL).

Tumor necrosis factor-alpha (TNF-α) was assayed in plasma by using specific enzyme-linked immunoabsorbant assays (Quantikine; R&D Systems, Minneapolis, MN) according to the manufacturers’ instruction. The detection limits were 3 pg/mL. For determination of plasma protein C, recombinant mouse and rat protein C were prepared essentially previously (20), and mouse protein C was needed to obtain rabbit anti-mouse protein C polyclonal antibody that was cross-reactive to rat for use in a standard enzyme-linked immunosorbent assay. For determination of circulating protein C concentrations, plasma samples for each animal were diluted and assayed in triplicate using this enzyme-linked immunoabsorbant assay; recombinant rat protein C was used as the reference standard.
Measurements of extracellular glutamate, glycerol, lactate/pyruvate ratio, dopamine, and serotonin in the striatum

Each animal was anesthetized with sodium pentobarbital administered intraperitoneally. The animals were mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA) with the nose positioned 3.3 mm below the horizontal line. After a midline incision, the skull was exposed and a bur hole was made in the skull for the insertion of a dialysis probe (4 mm in length, CMA/12; Carnegie Medicine, Stockholm, Sweden). The microdialysis probe was stereotaxically implanted into the striatum according to the atlas and coordinates of Paxinos and Watson (19). According to the methods detailed previously (21), the microdialysis was perfused at 2.0 ul/min and the dialysates were sampled in microvials. The dialysates were collected every 10 min in a CMA/140 fraction collection (Carnegie Medicine). Aliquots of dialysates (5 ul) were injected onto a CMA 600 microdialysis analyzer (Carnegie Medicine) for measurement of lactate, glycerol, pyruvate, and glutamate. Aliquots of dialysates (5 ul) were injected onto an Eicom EP-300 Liquid Chromatograph (Kyoto, Japan) for measurement of dopamine and serotonin.

Measurements of CBF, brain PO2, and brain temperature

A 100-µm diameter thermocouple and two 230-µm fibers were attached to the oxygen probe. This combined probe measures oxygen, temperature, and microvascular blood flow. The measurement requires OxyLite and OxyFlo instruments; OxyLite 2000 (Oxford Optronix, Oxford, UK) is a two-channel device (measuring PO2 and temperature at two sites simultaneously), whereas OxyFlo 2000 is two-channel laser Doppler perfusion monitoring instrument. The OxyLite has been designed to operate in conjunction with the OxyFlo. The combination of these two instruments provides simultaneous tissue blood flow, oxygenation, and temperature
data. Under anesthesia, the animal was placed in a stereotaxic apparatus, and the combined probe was implanted into the striatum using the atlas and coordinates of Paxinos and Watson (19). The detailed procedures from measurement of brain temperature, PO$_2$, and temperature were described previously (21).

**Measurement of extracellular hydroxyl radicals**

The concentrations of hydroxyl radicals were measured by a modified procedure based on the hydroxylation of sodium salicylate by hydroxyl radicals, leading to the production of 2,3-DHBA and 2,5-DHBA (14,17). A Ringer’s solution containing 0.5 mM sodium salicylate was perfused through the microdialysis probe at a constant flow rate (1.2 ml/min). After 2 h of stabilization, the dialysates from the striatum were collected at 10 min intervals. An Alltima reverse-phase C18 column (BAS, 150X1 MM I.D., particle size 5 mm) was used to separate the DHBAs, and the mobile phase consisted of a mixture of 0.1 M chloroacetic acid, 26.87 nM disodium EDTA, 688.76 nM sodium octylsuefate, and 10% acetonitrite (pH 3.0). The retention time of 2,3-DHBA and 2,5-DHBA were 8.1 and 6.0 min.

**Determination of lipid peroxidation**

Lipid peroxidation was assessed by measuring the levels of malondialdehyde (MDA) with 2-thiobarbituric acid (TBA) to form a chromophore absorbing at 532 nm (15). About 0.1 g of tissue was homogenized with 1.5 mL of 0.1 M phosphate buffer at pH 3.5. The reaction mixture (0.2 mL of sample, 1.5 mL of 20% acetic acid, 0.2 mL of 8.1% sodium dodecylsulfate, and 1.5 mL of aqueous solution of 0.8% TBA, up to 4 mL with distilled water) was heated to 95°C for 1h and then 5 mL of N-butanol and pyridine (15:1 v/v) was added. The mixture was vortexed vigorously, centrifuged at 1500 x g for 10 min and the absorbance of the organic phase measured at 532 nm. The values were expressed as nanomoles of TBA-reactive substances (MDA equivalent)
per milligram of protein.

**Quantification of total and oxidized glutathione**

Tissues were homogenized in 5% 5-sulfosalicylic acid (1:10 w/v) at 0°C, and the supernatants were used to analysis of total and oxidized glutathione. Total GSH (GSH+GSSG) was analyzed according to Tietze method (22) and oxidized GSH was determined as described by Griffith (23). The recycling assay for total GSH is oxidized by 5,5'-Dithiobis[2-nitrobenzoic acid] (DTNB) to give GSSG with spectrometric formation of 5-thio-2-nitro-benzoic acid (TNB). GSSG is reduced to GSH by the action of the highly specific glutathione reductase and NADPH. The rate of TNB formation is followed at 412 nm and is proportional to the sum of GSH and GSSG present.

**Monitoring of GPx and GR activity**

To measure cytosolic GPx and GR activities, tissues were homogenized in buffer solutions containing 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, and 1 mM dithiothreitol (DTT). The homogenates were centrifuged at 8500 x g for 15 min at 4°C, and supernatants were used for GPx and GR activity assay. The GPx and GR activities were performed with a commercial glutathione peroxidase cellular activity assay kit (Oxis Research, Portland, USA) and a glutathione reductase assay kit (Oxis Research, Portland, USA), respectively. One unit of GPx or GR activity was defined as the amount of sample required to oxidize 1 µmol of NADPH per minute based on the molecular absorbance of 6.22x10^6 for NADPH.

**Neuronal damage score**

At the end of the experiments, animals were killed by overdose of sodium pentobarbital and the brains were fixed in situ and left in the skull in 10% neutral buffered formation for at least 24 h before removal from the skull. The brain was
removed and embedded in paraffin blocks. Serial (10 um) sections through different brain structures including the hypothalamus, the hippocampus, the striatum, the frontal cortex, the cerebellum, and the brain stem were stained with hematoxylin and eosin for microscopic examination. The extent of cerebral neuronal damage was scored on a scale of 0 to 3 (24). According to the grading system, 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 without the examiner knowing the experimental conditions. When examined for neuronal damage in gray matter, only areas other than those invaded by probes were assessed.

**Superoxide dismutase (SOD) and Catalase activity monitoring**

To measure cytosolic SOD and catalase activities, tissues were homogenized in 200 mM phosphate buffer, pH 7.0. The homogenates were centrifuged at 3000 x g for 10 min at 4°C, and supernatants were used for SOD and catalase activity assay. The SOD and catalase activity were performed with a SOD activity commercial kit (Oxis Research, Portland, USA) and a catalase activity commercial kit (Oxis Research, Portland, USA), respectively. The SOD activity is determined from the ratio of the autoxidation rates in the presence (Vs) and in the absence (Vc) of SOD. One SOD activity unit is defined as the activity that doubles the autoxidation rate of the control bland (Vs/Vc = 2). The catalase activity is determined from the catalase standard curve (Oxis Research, Portland, USA). Protein concentration was determined by the method of Lowry et al (25).

**Statistical Method**

Data are presented as the mean ± SEM. For the data presented in Table 1, Table 2, Table 3, Table 4, Fig. 2, Fig. 3, Fig. 4, Fig. 5, Fig. 6, Fig. 7, Fig. 8 and Fig. 9,
Kruskal-Wallis H test was used for factorial experiments, whereas Dunn test was used for post hoc multiple comparisons among means. Wilcoxon tests were used for evaluation of neuronal damage scores in Table 5. Wilcoxon tests convert the scores or values of a variable to ranks require calculation of a sum of the ranks, and provide critical values for the sum necessary to test the null hypothesis at a given significant level. These data were presented as “median”, followed by first (Q1) and third (Q3) quartile. $P < 0.05$ was considered evidence of statistical significance.
RESULTS

**HRJVF improves survival during heat stroke**

Table 1 summarizes the survival time values for normothermic controls, heat stroke rats without treatment, or heat stroke rats with treatment (36°C, 24°C or 4°C saline 1.7 mL per 100 g of body weight, iv). The survival time values during heat stroke for rats without treatment were decreased from the control values of 477-483 min to new values of 21-25 min. Resuscitation with HRJVF of 4 or 24°C, but not 36°C saline 1.7 mL per 100 g of body weight increased the survival time values (56 to 230 min) during heat stroke. There was an insignificant change in body weight during the whole course of experiment for all heat stressed animals treated with HRJVF of 4, 24, or 36°C saline (data are not shown here).

**HRJVF attenuates heat stroke-induced physiologic dysfunction**

Figures 2 and 3 depict the effects of heat exposure (43°C for 70 min) on several physiologic and biochemical parameters in rats treated with 36°C saline, rats treated with HRJVF (4°C saline), and normothermic controls. In 36°C saline-treated heat stroke groups, the ICP, brain temperature, Tco and cellular levels of glutamate, glycerol, lactate/pyruvate, DHBA, dopamine, and serotonin in striatum were all significantly higher at 80 to 100 min after the start of heat exposure than they were for normothermic controls. In contrast, the values for MAP, cerebral perfusion pressure, CBF, brain PO₂, and HR were significantly lower than those of normothermic controls. Resuscitation with HRJVF (or 4°C saline) 70 min after initiation of heat exposure (or immediately at the time point of onset of heat stroke) significantly attenuated the heat stress-induced arterial hypotension, intracranial hypertension, cerebral hypoperfusion, cerebral hypoxia, hyperthermia, and increased levels of DHBA, dopamine, serotonin, glycerol, glutamate, and lactate/pyruvate ratio in striatum. The basal levels of
physiologic and biochemical parameters measured in normothermic rats treated with HRJVF (or 4°C saline) were indistinguishable from those of normothermic rats received no treatment (data not shown).

**HRJVF attenuates heat stroke-induced oxidative stress**

As shown in Figures 4-9, 15 min after the onset of heat stroke, in the 36°C saline-treated heatstroke group (HS), all the malondialdehyde (MDA) and GSSH/GSH ratio values obtained for different brain structures were significantly greater than those of normothermic controls (NC) ($P < 0.05$). In contrast, all the glutathione reductase, glutathione peroxidase, catalase, and SOD values obtained for different brain structures were significantly lower compared with control. Heatstroke-induced increased values of MDA and GSSG/GSH ratio as well as the decreased values of glutathione reductase, glutathione peroxidase, catalase and SOD in different brain structures were significantly attenuated by HRJVF (4°C saline) adopted immediately after the onset of heatstroke in (HS+J) group ($P < 0.05$).

**HRJVF does not improve heatstroke-induced hyperkalemia**

Table 2 summarizes the plasma levels of hemoglobin, hematocrit, Ca$^{2+}$, Na$^+$, K$^+$, Cl$^-$, Mg$^{2+}$ and myoglobin for normothermic controls, 36°C saline-treated heat stroke rats, and 4°C saline-treated heat stroke rats. It can be seen from the table that beside the plasma levels of K$^+$, all other parameters were not affected by heat stress. In addition, the heat stroke-induced hyperkalemia was not significantly reduced by HRJVF.

**HRJVF attenuates heat stroke-induced hypercoagulable state and TNF-α overproduction**

Table 3 summarizes the plasma levels of activated partial thromboplastin time (aPTT), prothrombin time (PT), platelet count, D-dimer, protein C, and TNF-α for
normothermic controls, 36°C saline-treated heat stroke rats, and 4°C saline-treated heat stroke rats. The table reveals that aPTT, PT, D-dimer and TNF-α values during heat stroke for rats resuscitated with 36°C saline were all significantly higher at 70 to 85 min after the start of heat exposure than they were for normothermic controls. In contrast, the values for plasma protein C and platelet count levels were all significantly lower than those of normothermic controls. Resuscitation with 4°C saline 70 min after initiation of heat exposure (or immediately at the onset of heat stroke) significantly attenuated the heat stress-induced increased plasma levels of aPTT, PT, D-dimers, and TNF-α, as well as the decreased plasma levels of protein C and platelet count. The plasma levels of aPTT, PT, platelet count, protein C, D-dimer, and TNF-α measured for normothermic rats treated with 4°C saline were indistinguishable from those of the normothermic rats without treatment.

**HRJVF attenuates heat stroke-induced cellular injury and organ dysfunction**

Table 4 summarizes the blood urea nitrogen (BUN) values and plasma levels of creatinine, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and alkaline phosphatase (ALP) for normothermic controls, 36°C saline-treated heat stroke rats, and 4°C saline-treated heat stroke rats. It can be seen from the table that the blood urea nitrogen values and plasma levels of creatinine, SGOT, SGPT, and ALP for 36°C saline-treated rats were all significantly higher at 70 to 85 min after the start of heat exposure than they were for normothermic controls. Resuscitation with 4°C 70 min after initiation of heat exposure significantly attenuated the heat stress-induced increased plasma levels of blood urea nitrogen, creatinine, SGOT, SGPT, and ALP. The blood urea nitrogen values and plasma levels of creatinine, SGOT, SGPT, and ALP measured for normothermic rats treated with 4°C saline were indistinguishable from those of the normothermic rats without treatment.
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**HRJVF attenuates heatstroke-induced neuronal damage**

Table 5 summarizes the neuronal damage scores of different brain structures in normothermic rats, 36°C saline-treated heatstroke rats, and HRJVF or 4°C saline-treated heatstroke rats. It was found that the scores for neuronal damage in heatstroke rats with 36°C saline via the jugular vein immediately after the time point of heatstroke onset or no treatment (median[Q₁, Q₃], [2,2]) were all significantly greater ($P < 0.05$) than those for the normothermic controls (median [Q₁, Q₃], 0 [0,0]). However, the neuronal damage scores for heatstroke rats treated with HRJVF (median [Q₁, Q₃], 0[0,1]) were all significantly lower ($P < 0.05$) than those for the heatstroke controls. Damaged neurons appeared shrunken with structureless and/or eosinophilic cytoplasm and shrunken nucleus (3, 4). HRJVF significantly reduced the neuronal damage.
DISCUSSION

Both of our previous (3) and present results showed that all heat-stressed animals displayed systemic inflammation and activated coagulation, evidenced by increased tumor necrosis factor-α, PT, aPTT, and D-dimer, and decreased platelet count and protein C. Biochemical markers evidenced cellular ischemia and injury/dysfunction: plasma levels of BUN, creatinine, SGOT, SGPT, and ALP, and striatal levels of glycerol, glutamate, lactate/pyruvate ratio, DHBA, lipid peroxidation, GSSG/GSH ratio, dopamine, and serotonin were all elevated during heat stroke. Both core and body temperatures and ICP were also increased during heat stroke. In contrast, the values of mean arterial pressure, cerebral perfusion pressure, and striatal levels of local blood flow, partial pressure of O₂, total superoxide dismutase, catalase, GPx, and GR activities were all significantly lower during heat stroke. The present findings further demonstrate that the circulatory dysfunction, systemic inflammation, hypercoagulable state, and cerebral oxidative stress, ischemia and damage occurring during heat stroke can be all significantly suppressed by brain cooling therapy induced by HRJVF.

According to an anatomy of brain in the laboratory rat (26), the relatively small internal jugular vein collects blood from the cerebral sinuses, whereas the larger external jugular vein is formed by fusion of the linguo-facial and maxillary vein. Since the internal jugular vein is too small to be cannulated for experimentation, the external jugular vein is chosen for cannulation in the present study. Thirty minutes after 5 mL of retrograde ice saline was infused via the external jugular vein, there was a decrease of 1.6°C in the striatum of brain (as shown in Figure 2).

An overview of the hypothesis of how heat stress leads to multiple organ injury and circulatory dysfunction has been proposed (27). At the whole organism level, heat
stress increases cutaneous blood flow and metabolism and progressively reduces splanchnic blood flow. Increased metabolic demand coupled with reduced splanchnic blood flow generates cellular ischemia and hypoxia, compromises cellular energy production, and produces homeostatic derangements. As heat stress continues, reactive oxygen species (ROS) generation can produce multifocal cellular injury and inflammation. Damage to the intestine increases intestinal permeability to endotoxins, contributing to local tissue inflammation and inducible nitric oxide synthase (iNOS) activation. The iNOS activation promotes reactive nitrogen species (RNS) generation. This inappropriate production of ROS and RNS causes splanchnic dilation, systemic hypotension, and circulatory shock. Inducible NOS under the pathological condition can be expressed in most tissues including neurons, astrocytes, and endothelial cells (28). In rat brain, iNOS protein was detectable after cerebral ischemia produced by middle cerebral artery ligation (29) or heatstroke (30). In addition, the heatstroke-induced cerebral ischemia and iNOS-dependent NO overproduction can be suppressed by pretreatment with aminoguanidine an iNOS inhibitor (30). The activation of the NMDA receptors and formation of NO by iNOS may directly signal the mitochondrial release of cytochrome c or formation of peroxynitrite (ONOO⁻), and subsequently hydroxyl radical production can directly damage lipids, proteins, and DNA and lead to cell death, most likely necrosis (31). Indeed, it has been recently confirmed that during heatstroke the tissue ischemic damage is associated with iNOS-dependent NO and peroxynitrite production (32).

In addition, as shown in our previous (15, 18, 33) and present findings, increased production of DHBA, lipid peroxidation, superoxide anion radicals (O₂⁻), MDA, GSSG/GSH ratio, and extracellular ischemia and damage markers in different brain structures (including the hypothalamus, striatum, cortex, hippocampus, and
cerebellum) all occurred during heatstroke. By contrast, decreased activity of glutathione reductase, glutathione peroxidase, catalase, and SOD in different brain structures were observed during heatstroke. During heatstroke, these endogenous antioxidative defenses are likely to be perturbed as a result of overproduction of oxygen radicals by cytosolic pro-oxidant enzymes, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in heat-injured (because of hyperthermia) and ischemic (because of ensuing arterial hypotension and cerebral hypoperfusion) brain tissues. These reactive oxygen species have been reported to be directly involved in oxidative damage with cellular macromolecules in ischemic tissues, which lead to cell death (29). Putting these observation together, it appears that heat stress causes heat stroke formation by inducing oxidative stress in both the periphery and the central nervous system.

In the present results, hypothermic retrograde jugular vein flush induced brain cooling in heatstroke rats, and resulted in reduction in oxidative stress, systemic inflammation, and activated coagulation, which led to attenuation of tissue ischemia and injury/dysfunction. Heat shock preconditioning (15) has also been shown to be able to ameliorate heatstroke-induced arterial hypotension and cerebral ischemia and damage in the rat by reducing hyperthermia as well as oxidative stress. These observations prompted us to think that although a reduction in the metabolic rate by induced hypothermia is believed to be the major mechanism by which therapeutic benefits are achieved (6, 7), other mechanisms such as suppressing oxidative stress, systemic inflammation and activated coagulation resulting in reduced tissue damage (8-14) may also be important.

The strength of conclusions could be significantly increased with additional groups using specific therapeutical interventions. Indeed, pretreatment with
conventional hydroxyl radical scavengers (e.g., mannitol, α-tocopherol or magnolol) prevented increased production of hydroxyl radicals, increased levels of lipid peroxidation, and ischemic neuronal damage in different brain structures attendant with heat stroke and increased subsequent survival time (33, 34). Shengmai San (a Chinese herbal medicine) or hypervolemic hemodilution was also shown to be able to prevent or repair ischemic injury in the brain during heat stroke by reducing oxidative stress (35, 36).

In our rat model, overproduction of cytokines including interleukin-1β (IL-1β), tumor necrosis factor-alpha (TNF-α), and IL-6 in the plasma occurs during heatstroke (33, 37, 38). The circulatory shock occurred during heatstroke can be mimicked by intravenous infusion of IL-1β (37). Previous antagonist of IL-1β receptor (37) are able to improve survival during heatstroke by reducing arterial hypotension and cerebral ischemia and damage (37). The present results further showed that HRJVF improved circulatory shock and cerebral ischemia and injury by reducing both inflammatory activation and hypercoagulable state during heat stroke. Therefore, we conclude that HRJVF may exert its therapeutic benefits by suppressing both cytokine overproduction and hypercoagulable state during heat stroke. In fact, a growing body of evidence has suggested that hypothermia blunts the inflammatory response and reduced organ injury after hemorrhage (39) as well as traumatic brain injury in the rat (40).

Other evidence has accumulated to suggest that marked activation of the nigrostriatal dopamine pathway is associated with ischemic neuronal injury during heatstroke (16, 41, 42). Destruction of central dopaminergic pathway protects against ischemic neuronal injury in experimental heatstroke (42). Heat shock protein 72 preconditioning also protects against ischemic insults during heatstroke by reducing
overload of cerebral dopamine (17). It has been implicated that sustained auto-oxidation of dopamine could lead to an excessive accumulation of cytotoxic oxygen free radicals (43). Whole body (20) or brain cooling (present results) may attenuate the oxidative stress during heatstroke by attenuating the overproduction of dopamine in the brain.

After the onset of heatstroke, rats without brain 5-HT depletion displayed hyperthermia, circulatory shock, intracranial hypertension, cerebral hypoperfusion, cerebral ischemia and injury in association with an increase in brain serotonin content (44). However, when the brain serotonin was destroyed by 5,7-dihydroxytryptamine, these heatstroke reactions were suppressed. A more recent report further showed that previous antagonism of 5-HA1A receptors or antagonism of 5-HA2A receptors protect against heatstroke by reducing circulatory shock and cerebral ischemia (45). In the present results, HRJVF may have attenuated oxidative stress during heatstroke by reducing overproduction of serotonin in the brain.

For human reasons, the present studies were conducted in rats under sodium pentobarbital anesthesia. Anesthesia causes impairment of normal body temperature regulation and has potential effects on the study of heat stroke pathophysiology in this model. Nevertheless, this potential source of variation should have been accounted for by the appropriate controls in the present study.

When the unanesthetized mice were exposed to heat stress, an amount of ~13% of body weight was lost after reaching a core temperature of 39.5°C (46). This body weight loss, which served as an indirect measure of dehydration, is not observable in our anesthetized, heat stressed rats resuscitated with an intravenous infusion of 1.7 mL per 100 g of body weight of normal saline over 5 min. In addition, our heat stressed rats did not display hypovolemia during the experimentation. It is believed that
dehydration and hypovolemia impair cardiovascular and thermoregulatory adjustments to heat stress (47).

Figure 10 shows a scheme depicting events between the exposure of rats to a hot environment and death from heatstroke, with known or proposed interrelationships derived from the published data. After the onset of heat stroke, cessation or reduction of blood flow to the brain (resulting from both arterial hypotension and intracranial hypertension) induces neuronal damage. This neurotoxic cascade involves overproduction of glutamate, dopamine, serotonin, and cytokines as well as oxidative stress in the brain. Any measures which are able to restore cerebral blood flow and to intervene the secondary neurotoxic cascades can be used to limit cerebral neuronal damage and death in heat stroke. In the periphery, arterial hypotension causes tissue ischemia and hypoxia and leads to oxidative stress in multiple organs. Both systemic inflammation and hypercoagulable state resulting from oxidative stress may contribute to multiple organ failure and dysfunction. Again, any measures which are able to main appropriate levels of mean arterial pressure and to intervene the tissue ischemia can be used to limit oxidative stress in the peripheral tissues.
REFERENCES


10. Maier CM, Sun GH, Cheng D, Yenari MA, Chan PH and Steinberg GK: Effects of mild hypothermia on superoxide anion production, superoxide dismutase expression, and activity following transient focal cerebral ischemia. Neurobiol Dis


47. Massett MP, Johnson DG, Kregel KC: Cardiovascular and sympathoadrenal
TABLE 1. Survival time values for normothermic controls and heatstroke rats without or with cold saline delivery via jugular vein

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Survival time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normothermic controls (no treatment)</td>
<td>480±3(8)</td>
</tr>
<tr>
<td>2. Heatstroke rats (no treatment)</td>
<td>23±2(8)</td>
</tr>
<tr>
<td>3. Heatstroke rats (treatment with 36°C saline 1.7 mL per 100 g of body weight, iv)</td>
<td>25±3(8)*</td>
</tr>
<tr>
<td>4. Heatstroke rats (treatment with 24°C saline 1.7 mL per 100 g of body weight, iv)</td>
<td>56±7(8)†</td>
</tr>
<tr>
<td>5. Heatstroke rats (treatment with 4°C saline 1.7 mL per 100 g of body weight, iv)</td>
<td>230±22(8)†</td>
</tr>
</tbody>
</table>

* P < 0.05 in comparison with group 1; † P < 0.05 in comparison with group 3

(Kruskal-Wallis H test followed by Dunn test). All heatstroke rats had exposure (43°C) withdrawn exactly at 70 min and were then allowed to recover at room temperature (24°C). Data are mean ± SEM, followed by number of animals (n) in parentheses. Group 1 was killed approximately 480 min after the initiation of experimentation (or at the end of the experiments) with an overdose of sodium pentobarbital.
TABLE 2. The plasma levels of hemoglobin (Hb), hematocrit (Hct), Ca^{2+}, Na^{+}, Cl^{-}, Mg^{2+}, and myoglobin for normothermic controls (NC), 36°C saline-treated heatstroke rats (HS), and 4°C saline-treated heat stroke rats (HS+J)

<table>
<thead>
<tr>
<th>Treatment group/Time course</th>
<th>Hb (g/dL)</th>
<th>Hct (%)</th>
<th>Ca^{2+} (mg/dL)</th>
<th>Na^{+} (mEg/L)</th>
<th>K^{+} (mEg/L)</th>
<th>Cl^{-} (mEg/L)</th>
<th>Mg^{2+} (mg/dL)</th>
<th>Myoglobin (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC: 0 min</td>
<td>15.2±0.1</td>
<td>43.9±1.2</td>
<td>10.8±0.3</td>
<td>142±7</td>
<td>4.5±0.2</td>
<td>104±1</td>
<td>2.1±0.1</td>
<td>1.0±0</td>
</tr>
<tr>
<td>70 min</td>
<td>15.1±0.2</td>
<td>43.8±1.4</td>
<td>10.7±0.2</td>
<td>141±6</td>
<td>4.6±0.1</td>
<td>103±0</td>
<td>2.0±0</td>
<td>1.0±0</td>
</tr>
<tr>
<td>85 min</td>
<td>15.3±0.3</td>
<td>43.9±1.3</td>
<td>10.7±0.1</td>
<td>142±5</td>
<td>4.6±0.2</td>
<td>104±1</td>
<td>2.0±0</td>
<td>1.0±0</td>
</tr>
<tr>
<td>HS: 0 min</td>
<td>15.2±0.1</td>
<td>43.8±1.3</td>
<td>10.9±0.2</td>
<td>142±6</td>
<td>4.4±0.2</td>
<td>104±1</td>
<td>2.2±0.1</td>
<td>1.0±0</td>
</tr>
<tr>
<td>70 min</td>
<td>15.3±0.6</td>
<td>46.3±1.8</td>
<td>10.7±0.2</td>
<td>147±7</td>
<td>8.4±0.8*</td>
<td>103±1</td>
<td>2.1±0.3</td>
<td>1.0±0</td>
</tr>
<tr>
<td>85 min</td>
<td>15.4±0.9</td>
<td>46.0±2.7</td>
<td>10.8±0.3</td>
<td>143±6</td>
<td>8.5±0.5*</td>
<td>103±0</td>
<td>2.1±0.2</td>
<td>1.0±0</td>
</tr>
<tr>
<td>HS+J: 0 min</td>
<td>15.3±0.2</td>
<td>44.8±1.4</td>
<td>10.7±0.1</td>
<td>143±6</td>
<td>4.6±0.1</td>
<td>104±1</td>
<td>2.2±0.1</td>
<td>1.0±0.2</td>
</tr>
<tr>
<td>70 min</td>
<td>15.8±0.6</td>
<td>46.7±2.0</td>
<td>10.5±0.3</td>
<td>142±7</td>
<td>8.2±0.7*</td>
<td>103±1</td>
<td>2.1±0.1</td>
<td>1.0±0</td>
</tr>
<tr>
<td>85 min</td>
<td>15.2±0.1</td>
<td>45.4±1.5</td>
<td>10.6±0.2</td>
<td>146±7</td>
<td>7.6±0.6*</td>
<td>107±2</td>
<td>2.1±0.5</td>
<td>1.0±0</td>
</tr>
</tbody>
</table>

*P<0.05 in comparison with group 1 (Kruskal-Wallis H test followed by Dunn test). All HS and HS+J groups had heat exposure (43°C) withdrawn exactly at 70 min and were then allowed to recover at room temperature (24°C). Data are mean±SEM of eight rats per group. The samples were obtained 0, 70, or 85 min after the initiation of heat exposure in heat stroke rats or the equivalent times in normothermic controls.
<table>
<thead>
<tr>
<th>Treatment group/Time course</th>
<th>aPTT(s)</th>
<th>PT(s)</th>
<th>Protein C (µg/L)</th>
<th>D-dimer (µg/L)</th>
<th>TNF-α (pg/mL)</th>
<th>Platelet counts (×1000/µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC: 0 min</td>
<td>25±2</td>
<td>8.5±0.1</td>
<td>3.1±0.1</td>
<td>47±3</td>
<td>21±3</td>
<td>1111±79</td>
</tr>
<tr>
<td>70 min</td>
<td>26±3</td>
<td>8.7±0.2</td>
<td>3.0±0.1</td>
<td>45±2</td>
<td>20±4</td>
<td>1115±81</td>
</tr>
<tr>
<td>85 min</td>
<td>24±3</td>
<td>8.9±0.3</td>
<td>3.0±0.1</td>
<td>46±3</td>
<td>22±3</td>
<td>1119±75</td>
</tr>
<tr>
<td>HS: 0min</td>
<td>23±2</td>
<td>8.8±0.2</td>
<td>3.1±0.1</td>
<td>46±2</td>
<td>23±4</td>
<td>1115±83</td>
</tr>
<tr>
<td>70 min</td>
<td>95±10*</td>
<td>13.5±0.2*</td>
<td>2.0±0.1*</td>
<td>92±4*</td>
<td>798±11*</td>
<td>778±66*</td>
</tr>
<tr>
<td>85 min</td>
<td>106±11*</td>
<td>14.3±0.3*</td>
<td>0.6±0.1*</td>
<td>118±7*</td>
<td>886±15*</td>
<td>746±58*</td>
</tr>
<tr>
<td>HS+J: 0 min</td>
<td>24±2</td>
<td>9.0±0.2</td>
<td>3.0±0.1</td>
<td>46±3</td>
<td>22±3</td>
<td>1109±78</td>
</tr>
<tr>
<td>70 min</td>
<td>93±9</td>
<td>14.2±0.4</td>
<td>2.0±0.1</td>
<td>90±4</td>
<td>801±12</td>
<td>805±72</td>
</tr>
<tr>
<td>85 min</td>
<td>44±5†</td>
<td>9.6±0.3†</td>
<td>2.0±0.1†</td>
<td>68±4†</td>
<td>345±15†</td>
<td>1088±55†</td>
</tr>
</tbody>
</table>

*P<0.05 in comparison with group 1; †P<0.05 in comparison with group 2 (Kruskal-Wallis H test followed by Dunn test). All HS and HS+J groups had heat exposure (43°C) withdrawn exactly at 70 min and were then allowed to recover at room temperature (24°C). Data are mean±SEM of eight rats per group. The samples were obtained 0, 70, 85 min after the initiation of heat exposure in heat stroke rats or the equivalent times in normothermic controls.

TABLE 3. The plasma levels of aPTT, PT, protein C, D-dimer, TNF-α and platelet counts for normothermic controls (NC), 36°C saline-treated heat stroke rats (HS), and 4°C saline-treated heat stroke rats (HS+J)
**TABLE 4.** The plasma levels of creatinine, BUN, SGPT, SGOT, and ALP for normothermic controls (NC), 36°C saline-treated heat stroke rats (HS), and 4°C saline-treated heat stroke rats (HS+J)

<table>
<thead>
<tr>
<th>Treatment group/Time course</th>
<th>Creatinine (mg/dL)</th>
<th>BUN (mg/dL)</th>
<th>SGPT (1 U/L)</th>
<th>SGOT (1 U/L)</th>
<th>ALP (1 U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC: 0 min</td>
<td>0.42±0.02</td>
<td>14±1</td>
<td>80±5</td>
<td>162±18</td>
<td>459±42</td>
</tr>
<tr>
<td>70 min</td>
<td>0.45±0.03</td>
<td>15±2</td>
<td>82±7</td>
<td>158±17</td>
<td>463±43</td>
</tr>
<tr>
<td>85 min</td>
<td>0.46±0.02</td>
<td>13±2</td>
<td>79±6</td>
<td>163±16</td>
<td>467±45</td>
</tr>
<tr>
<td>HS: 0 min</td>
<td>0.43±0.03</td>
<td>15±2</td>
<td>81±6</td>
<td>172±19</td>
<td>448±39</td>
</tr>
<tr>
<td>70 min</td>
<td>0.97±0.07*</td>
<td>28±3*</td>
<td>228±17*</td>
<td>476±21*</td>
<td>645±49*</td>
</tr>
<tr>
<td>85 min</td>
<td>1.25±0.28*</td>
<td>33±2*</td>
<td>247±19*</td>
<td>511±28*</td>
<td>773±45*</td>
</tr>
<tr>
<td>HS+J: 0 min</td>
<td>0.44±0.04</td>
<td>14±2</td>
<td>79±7</td>
<td>168±19</td>
<td>451±41</td>
</tr>
<tr>
<td>70 min</td>
<td>0.99±0.09</td>
<td>26±2</td>
<td>225±18</td>
<td>469±22</td>
<td>652±48</td>
</tr>
<tr>
<td>85 min</td>
<td>0.51±0.07†</td>
<td>22±1†</td>
<td>106±21†</td>
<td>195±20†</td>
<td>526±39†</td>
</tr>
</tbody>
</table>

*P*<0.05 in comparison with group 1; †*P*<0.05 in comparison with group 2 (Kruskal-Wallis H test followed by Dunn test). All HS and HS+J group had heat exposure (43°C) withdrawn exactly at 70 min and were then allowed to recover at room temperature (24°C). Data are mean±SEM of eight rats per group. The samples were obtained 0, 70, 85 min after the initiation of heat exposure in heat stroke rats or the equivalent times in normothermic controls.
**Table 5.** Effect of heat stress (ambient temperature of 43°C for 70 min) on neuronal damage in different brain structures of normothermic controls, 36°C saline-treated rats, or 4°C saline-treated heatstroke rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hypothalamus</th>
<th>Striatum</th>
<th>Hippocampus</th>
<th>Cortex</th>
<th>Cerebellum</th>
<th>Brain stem</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normothermic controls (no treatment)</td>
<td>0(0.0)</td>
<td>0(0.0,0.75)</td>
<td>0(0.0)</td>
<td>0(0.0,0.75)</td>
<td>0(0.0)</td>
<td>0(0.0,0.75)</td>
</tr>
<tr>
<td>2. Heatstroke rats (no treatment)</td>
<td>2(2,2)*</td>
<td>2(2,2)*</td>
<td>2(2,2)*</td>
<td>2(2,2)*</td>
<td>2(2,2)*</td>
<td>2(2,2)*</td>
</tr>
<tr>
<td>3. Heatstroke rats (treatment with 36°C saline 1.7 mL per 100 g of body weight, iv)</td>
<td>2(2,2)*</td>
<td>2(2,2)*</td>
<td>2(2,2)*</td>
<td>2(2,2)*</td>
<td>2(2,2)*</td>
<td>2(2,2)*</td>
</tr>
<tr>
<td>4. Heatstroke rats (treatment with 4°C saline 1.7 mL per 100 g of body weight, iv)</td>
<td>0(0.1)†</td>
<td>0(0.1)†</td>
<td>0(0.1)†</td>
<td>0(0.1)†</td>
<td>0(0.1)†</td>
<td>0(0.1)†</td>
</tr>
</tbody>
</table>

Data for eight rats per group presented as “median” with Q1 and Q3” in parentheses. For the determination of neuronal damage score, animals were killed 90 min after the onset of heat exposure or at the equivalent time for the normothermic controls. * P < 0.05 in comparison with group 1; † P < 0.05 in comparison with group 2 (Wilcoxon test). All heatstroke rats had heat exposure withdrawn exactly at 70 min and were then allowed to recover at room temperature (24°C). 36°C or 4°C saline were administered 70 min after initiation of heat exposure via the jugular vein.
**FIG. 1.**

Heat stress (
(Heat on)
)

Biochemical assay

Biochemical & histological assay

NC:

24°C

0' 70' 85'

24°C

24°C

24°C

24°C

HS:

24°C

0'

43°C

(Heat on)

Heat stress

70'

(Heat off)

36°C saline treatment

24°C

24°C

24°C

85'

HS+J:

24°C

0'

43°C

(Heat on)

Heat stress

70'

(Heat off)

24 or 4°C saline treatment

24°C

24°C

24°C

85'
FIG. 2.
FIG. 3.
FIG. 4.
Glutathione Peroxidase Activity (mU/mg prot.)

FIG. 5.
FIG. 6.
FIG. 7.
FIG. 8.
FIG. 9.
Hot environment

Anesthetized rats

↑ Tco, ↑ Tbr

↓ MAP

Tissue ischemia & hypoxia

Oxidative stress in the periphery

Systemic inflammation
Hypercoagulable state

Multiple organ failure

↓ CPP

Cerebral ischemia
Cerebral hypoxia

Oxidative stress in brain
1. pro-oxidant stimulation
   ↑ DHBA,
   ↑ lipid peroxidation
   ↑ GSSG/GSH ratio
2. anti-oxidant inhibition
   ↓ superoxide dismutase
   ↓ catalase
   ↓ GPx
   ↓ GR

↑ Glutamate
↑ Monoamines
↑ Cytokines

↓ ICP

Neuronal damage
CNS dysfunction
Heat stroke
Death

FIG. 10
The legends of the figures

FIG. 1. Experimental design.

The legends of the figures

FIG. 2. Effects of heat stress (ambient temperature [Ta] of 43°C for 70 min) on mean arterial pressure (MAP), intracranial pressure (ICP), cerebral perfusion pressure (CPP), cerebral blood flow (CBF), brain PO₂, and brain temperature (Tb). ○, values at Ta 43°C in eight rats treated with 36°C-normal saline (1.7 mL per 100 g of body weight, iv) immediately after the onset of heatstroke. Another eight rats exposed to a Ta of 24°C served as a control (▲). ●, values at Ta of 43°C in eight rats treated with 4°C-normal saline (1.7 mL per 100 g of body weight, iv) immediately after the onset of heatstroke. Points represent mean ± SEM (* P < 0.05 in comparison with normothermic control values [at Ta of 24°C]; † P < 0.05 in comparison with 36°C normal saline-treated group [at Ta of 43°C]; Kruskal-Wallis H test followed by Dunn test). The onset of heatstroke is indicated by the dashed line.

FIG. 3. Effects of heat stress (Ta of 43°C for 70 min) on core temperature (Tco), heart rate (HR), and extracellular levels of dihydroxybenzoic acid (DHBA), glutamate, glycerol, lactate/pyruvate, dopamine, and serotonin in the striatum. ○, values at ambient temperature (Ta) 43°C in eight rats treated with 36°C-normal saline (1.7 mL per 100 g of body weight, iv). Another eight rats exposed to a Ta of 24°C served as a control (▲). ●, values at Ta of 43°C in eight rats treated with 4°C-normal saline (1.7 mL per 100 mg of body weight, iv). The normal saline solution was administered immediately after the onset of heatstroke, as indicated by the dashed line (* P < 0.05 in comparison with normothermic control values [at Ta of 24°C]; † P < 0.05 in comparison with 36°C-normal saline-treated group [at Ta of 43°C]; Kruskal-Wallis H test followed by Dunn test).

FIG. 4. Effects of heat stress (ambient temperature [Ta] of 43°C for 70 min) on the
concentrations of malondialdehyde in the tissue homogenates of the hypothalamus (HT), cortex (CT), hippocampus (HC), striatum (ST), cerebellum (CB), and brain stem (BS) of normothermic controls (NC), heatstroke rats treated with 36°C-normal saline (HS), or heatstroke rats treated with 4°C-normal saline (HS+J). The normal saline solutions were administered via the jugular vein immediately after the onset of heatstroke (or 70 min after initiation of heat stress). The heatstroke rats were killed 90 min after the start of heat stress for determination of malondialdehyde in different brain structures. Values are means±SEM; n=8 per group. * P < 0.05 versus NC group; # P < 0.05 versus HS group (Kruskal-Wallis H test followed by Dunn test).

FIG. 5. Effects of heat stress (Ta of 43°C for 70 min) on the activities of glutathione peroxidase in the tissue homogenates of the hypothalamus (HT), cortex (CT), hippocampus (HC), striatum (ST), cerebellum (CB), and brain stem (BS) of normothermic controls (NC), heatstroke rats treated with 36°C-normal saline (HS), or heatstroke rats treated with 4°C-normal saline (HS+J). The normal saline solutions were administered via the jugular vein immediately after the onset of heatstroke (or 70 min after initiation of heat stroke). The heat-stressed rats were killed 90 min after the start of heat exposure for determination of glutathione peroxidase activity in different brain structures. Values are means±SEM; n=8 per group. * P < 0.05 versus NC group; # P < 0.05 versus HS group (Kruskal-Wallis H test followed by Dunn test).

FIG. 6. Effects of heat stress (Ta of 43°C for 70 min) on the activities of glutathione reductase in the tissue homogenates of the hypothalamus (HT), cortex (CT), hippocampus (HC), striatum (ST), cerebellum (CB), and brain stem (BS) of normothermic controls (NC), heatstroke rats treated with 36°C-normal saline (HS), or heatstroke rats treated with 4°C-normal saline (HS+J). The normal saline solutions were administered via the jugular vein immediately after the onset of heatstroke (or 70 min after initiation of heat stroke).
min after initiation of heat stroke). The heat-stressed rats were killed 85 min after the start of heat exposure for determination of glutathione reductase activity in different brain structures. Values are means ± SEM; n=8 per group. * \( P < 0.05 \) versus NC group; \# \( P < 0.05 \) versus HS group (Kruskal-Wallis H test followed by Dunn test).

FIG. 7. Effects of heat stress (Ta of 43°C for 70 min) on the levels of oxidized-form glutathione (GSSG)/reduced-form glutathione (GSH) ratio in the tissue homogenates of the hypothalamus (HT), cortex (CT), hippocampus (HC), striatum (ST), cerebellum (CB), and brain stem (BS) of normothermic controls (NC), heatstroke rats treated with 36°C-normal saline (HS), or heatstroke rats treated with 4°C-normal saline (HS+J). The normal saline solutions were administered via the jugular vein immediately after the onset of heatstroke (or 70 min after initiation of heat stroke). The heat-stressed rats were killed 85 min after the start of heat exposure for determination of GSSG/GSH ratio in different brain structures. Values are means ± SEM; n=8 per group. * \( P < 0.05 \) versus NC group; \# \( P < 0.05 \) versus HS group (Kruskal-Wallis H test followed by Dunn test).

FIG. 8. Effects of heat stress (Ta of 43°C for 70 min) on the activities of catalase in the tissue homogenates of the hypothalamus (HT), cortex (CT), hippocampus (HC), striatum (ST), cerebellum (CB), and brain stem (BS) of normothermic controls (NC), heatstroke rats treated with 36°C-normal saline (HS), or heatstroke rats treated with 4°C-normal saline (HS+J). The normal saline solutions were administered via the jugular vein immediately after the onset of heatstroke (or 70 min after initiation of heat stroke). The heat-stressed rats were killed 85 min after the start of heat exposure for determination of catalase activities in different brain structures. Values are means ± SEM; n=8 per group. * \( P < 0.05 \) versus NC group; \# \( P < 0.05 \) versus HS group (Kruskal-Wallis H test followed by Dunn test).
FIG. 9. Effects of heat stress (Ta of 43°C for 70 min) on the activities of superoxide dismutase (SOD) in the tissue homogenates of the hypothalamus (HT), cortex (CT), hippocampus (HC), striatum (ST), cerebellum (CB), and brain stem (BS) of normothermic controls (NC), heatstroke rats treated with 36°C-normal saline (HS), or heatstroke rats treated with 4°C-normal saline (HS+J). The normal saline solutions were administered via the jugular vein immediately after the onset of heatstroke (or 70 min after initiation of heat stroke). The heat-stressed rats were killed 85 min after the start of heat exposure for determination of SOD activities in different brain structures. Values are means ± SEM; n=8 per group. * P<0.05 versus NC group; # P<0.05 versus HS group (Kruskal-Wallis H test followed by Dunn test).

FIG. 10. Proposed scheme of the interacting sequence of events occurring from the beginning of exposure to a hot environment to heat stroke occurrence. Arrows indicate increased (↑) or decreased (↓). Tco, core temperature; Tbr, brain temperature; MAP, mean arterial pressure; ICP, intracranial pressure; CPP, cerebral perfusion pressure; CNS, central nervous system; DHBA, dihydroxy benzoic acid; GSSG, oxidized-form glutathione; GSH, reduced-form glutathione; GPx, glutathione peroxidase; GR, glutathione reductase.