Effect of hypervolaemic haemodilution on cerebral glutamate, glycerol, lactate and free radicals in heatstroke rats

Chen-Kuei Chang*, Wen-Ta Chu†, Ching-Ping Chang‡ and Mao-Tsun Lin‡

*Division of Neurosurgery, Department of Surgery, Mackay Memorial Hospital, Taipei, Taiwan, †Graduate Institute of Injury Prevention and Control, Taipei Medical University and Municipal Wan-Fan Hospital, Taipei, Taiwan, and ‡Department of Medical Research, Chi-Mei Medical Center, Tainan, Taiwan

ABSTRACT

In the present study, we attempted to assess the mechanisms underlying the neuroprotective effect of hypervolaemic haemodilution in rat heatstroke. In anaesthetized rats treated with normal saline (NS) immediately after the onset of heatstroke induced by $T_a$ (ambient temperature) of 42°C for 88 min, followed by $T_a$ of 24°C for 12 min, the values for MAP (mean arterial pressure), ICP (intracranial pressure), CPP (cerebral perfusion pressure), CBF (cerebral blood flow), brain $P_O_2$ (partial pressure of $O_2$) and striatal glutamate, glycerol, lactate/pyruvate ratio, hydroxyl radicals and neuronal damage score were 42 ± 3 mmHg, 33 ± 3 mmHg, 9 ± 3 mmHg, 109 ± 20 BPU (blood perfusion units), 6 ± 1 mmHg, 51 ± 7 µmol/l, 24 ± 3 µmol/l, 124 ± 32, 694 ± 22% of baseline and 2.25 ± 0.05 respectively. In animals treated with 10% albumin immediately after the onset of heatstroke ($T_a$ of 42°C for 88 min), the values for MAP, ICP, CPP, CBF, brain $P_O_2$ and striatal glutamate, glycerol, lactate/pyruvate ratio, hydroxyl radicals and neuronal damage score were 64 ± 6 mmHg, 10 ± 2 mmHg, 54 ± 5 mmHg, 452 ± 75 BPU, 15 ± 2 mmHg, 3 ± 2 µmol/l, 4 ± 2 µmol/l, 7 ± 3, 119 ± 7% of baseline and 0.38 ± 0.05 respectively. Apparently, the heatstroke-induced arterial hypotension, intracranial hypertension, cerebral hypoperfusion, cerebral ischaemia, brain hypoxia, increased levels of striatal glutamate, glycerol, lactate/pyruvate ratio and hydroxyl radicals, and increased striatal neuronal damage score values were all attenuated significantly by the induction of hypervolaemic haemodilution in rats immediately at the onset of heatstroke. These results demonstrate that the neuroprotective effect of hypervolaemic haemodilution is associated with a decrease in the elevation of glutamate, glycerol, lactate and free radicals in brain exposed to experimental heatstroke-induced cerebral ischaemia/hypoxia injury.

INTRODUCTION

Evidence has accumulated to indicate that heatstroke-induced cerebral ischaemia and neuronal damage is associated with an increased production of free radicals, specifically hydroxyl radicals and $O_2^{•−}$ (superoxide), higher lipid peroxidation and lower enzymic antioxidant defences [1]. Pretreatment with hydroxyl radical scavengers, such as mannitol and $\alpha$-tocopherol, prevents production of hydroxyl radicals, reduces lipid peroxidation and ischaemic neuronal damage in different brain areas of rats exposed to heatstroke and prolongs subsequent survival [2]. Our previous results [3] have demonstrated that extracellular levels of glutamate,
glycerol and lactate/pyruvate in ischaemic/hypoxic brain were greater in heatstroke rats compared with those of normothermic controls.

Other lines of evidence have also shown [4] that heatstroke-induced cerebral ischaemia and injury is greatly attenuated by induction of hypervolaemic haemodilution with intravenous administration of 10 % human albumin started either before the heat stress or right after the onset of heatstroke in rats. The present study attempted to ascertain whether the neuroprotective effect of hypervolaemic haemodilution therapy is associated with inhibition of cerebral release of glutamate, glycerol, lactate and free radicals after cerebral ischaemia in heatstroke rats. The extracellular concentrations of glutamate, glycerol, lactate and hydroxyl radicals in the corpus striatum of rat brain were assessed by intracerebral microdialysis methods.

**Materials and Methods**

**Experimental animals**

Adult Sprague–Dawley rats (weight, 275 ± 14 g) were obtained from the Animal Resource Center of the National Science Council of the Republic of China (Taipei, Taiwan). The animals (n = 4/group) were housed at a Ta 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). Adequate anaesthesia was maintained to abolish the corneal reflex and pain reflexes induced by tail pinching throughout all experiments (approx. 8 h) by a single intraperitoneal dose of urethane (1.4 g/kg of body weight). At the end of the experiments, control rats and any 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 (PE 50), under urethane anaesthesia, for blood pressure monitoring and drug administration. The animals were positioned in a stereotaxic apparatus (Kopf model 1406; Grass Instrument Co., Quincy, MA, U.S.A.) to insert probes for measurement of ICP (intracranial pressure). ICP was monitored with a Statham P23AC transducer via a 20-gauge stainless-steel needle probe (diameter, 0.90 mm; length, 38 mm; Gould Instruments, Cleveland, OH, U.S.A.), which was introduced into the right lateral cerebral ventricle according to the stereotaxic coordinates of Paxinos and Watson [5] (7.7 mm interaural, 2.0 mm from the mid-line, and 3.5 mm from the top of the skull). All recordings were made on a four-channel Gould polygraph. Hct (haematocrit) was measured via a blood gas analyser (Nova Biochemical, Waltham, MA, U.S.A.). Ta (colonic temperature) was monitored continuously by a thermocouple and both MAP (mean arterial pressure) and HR (heart rate) were monitored continuously with a pressure transducer. Tco was maintained at approx. 36 °C using the electric thermal mat before the start of the experiment.

**Experimental groups**

Animals were assigned randomly to the following five groups: normal saline (NS)-pretreated rats maintained at 24 °C; NS-pretreated rats maintained at 42 °C; albumin-pretreated rats maintained at 42 °C; NS-post-treated rats maintained at 42 °C; and albumin-post-treated rats maintained at 42 °C. In NS-pretreated rats at 24 °C, animals were exposed to Ta of 24 °C for at least 90 min to reach thermal equilibrium and injected with 0.9 % NaCl solution (10 ml/kg of body weight) before the start of the experiment. In NS-pretreated rats at 42 °C, animals were injected with 0.9 % NaCl solution (10 ml/kg of body weight) i.v. (intravenously) and exposed immediately to Ta of 42 °C (with a relative humidity of 60 % in a temperature-controlled chamber). The moment at which MAP and local CBF (cerebral blood flow) began to decrease from their peak levels was taken as the onset of heatstroke ([1,6], and see Figure 1). In albumin-pretreated rats at 42 °C, animals received 10 ml of 10 % human albumin/kg of body weight (Travenol Laboratories, Glendale, CA, U.S.A.) i.v. at the onset of heat exposure. In NS-post-treated rats at 42 °C, animals received 0.9 % NaCl solution (10 ml/kg of body weight) i.v. at the onset of heatstroke. In albumin-post-treated rats at 42 °C, animals received 10 ml of 10 % albumin/kg of body weight i.v. at the onset of heatstroke. Immediately after the onset of heatstroke in these groups of animals, the heat exposure was switched off. The latency for the onset of heatstroke (e.g. interval between the onset of heat exposure and the onset of heatstroke) was determined in a different set of animals. In the present study, all groups of rats were exposed to 42 °C until the onset of heatstroke. For determination of survival time (e.g. interval between the onset of heatstroke and cardiac arrest), different physiological parameter changes or mortality rate, the heat exposure was switched off at exactly 88 min in all groups of rats. The mortality rate was defined by the number of rats that died within 480 min (88 min of heat exposure plus the subsequent 392 min of room temperature exposure) divided by the total number of rats tested. Different group of animals were used for the different sets of experiments: (i) measurement of ICP, CPP, MAP and striatal CBF, PaO2 (partial pressure of O2) and temperature; (ii) determination of colonic temperature, MAP, HR and extracellular concentrations of glutamate, glycerol, lactate, pyruvate and lactate/pyruvate in the corpus striatum; (iii) determination of latency;
Measurement of extracellular glutamate, glycerol, lactate/pyruvate and hydroxy radicals in the corpus striatum

Animals were anaesthetized with urethane administered intraperitoneally. The animal’s head was mounted in a stereotaxic apparatus (Davis Kopf Instruments, Tujunga, CA, U.S.A.) with the nose bar positioned 3.3 mm below the horizontal line. Following a midline incision, the skull was exposed and a burr 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 corpus striatum, according to the atlas and coordinates of Paxinos and Watson [5]. The coordinates for the right corpus striatum were: 9.7 mm interaural, 2.0 mm from the midline, and 4.5 mm from the top of the skull. As described previously [7], an equilibrium period of 60 min without sampling was allowed after probe implantation. The microdialysis probes were perfused at 2 µl/min with a sterile isotonic solution containing 147 mmol/l Na+, 4.0 mmol/l K+, 2.3 mmol/l Ca2+ and 156 mmol/l Cl–, and the dialysates were sampled in microvials. The dialysates were collected every 10 min in a CMA/140 fraction collector. Aliquots of dialysates (5 µl) were injected into a CMA600 Microdialysis analyser for measurement of lactate, glycerol, pyruvate and glutamate. All reagents required for analysis were obtained from CMA Microdialysis.

The concentrations of hydroxyl radicals were measured by a modified procedure based on the hydroxylation of sodium salicylate by hydroxyl radicals, leading to production of 2,3-dihydroxybenzoic acid (2,3-DHBA) and 2,5-DHBA [8–10]. These two compounds obtained from CMA Microdialysis.

Measurement of lipid peroxidation

In a separate set of animals, 12 min after the onset of heatstroke each group of animals was decapitated. The corpus striatum was removed from the rat brain. A fluorescence assay procedure was used to measure lipid peroxidation in this brain area [11]. The brain area was homogenized with water for 2 s in an ultrasonic tissue disrupter. The homogenates were mixed with 400 µl of ice-cold chloroform and 200 µl of methanol and then vortex-mixed for 1 min and left on ice for 15 min. After centrifugation at 8000 g for 5 min, 400 µl of the chloroform layer was transferred to another tube containing 100 µl of methanol and was scanned with a spectrofluorometer. The lipid peroxidation was determined by measuring the levels of malondialdehyde and its dihydropyridine polymers at 356 nm excitation and 426 nm emission.

Isolation of mitochondria and preparation of submitochondrial particles

All steps in the isolation of mitochondria and the preparation of submitochondrial particles were performed at ice-cold temperature. Mitochondria from the brain were isolated as described by Ozawa et al. [12]. Briefly, the corpus striatum was dissected out, rinsed and homogenized in 10 vol. (w/v) of ice-cold isolation buffer [0.3 mol/l mannitol and 0.1 mmol/l EDTA (pH 7.4)]. The homogenate was centrifuged at 600 g for 10 min, and the supernatant was recentrifuged at 10 000 g for 10 min. The resulting pellet was resuspended in 40 ml of isolation buffer and centrifuged at 5000 g for 10 min. The pellet was resuspended in 30 mmol/l potassium phosphate buffer (pH 7.0).

Submitochondrial particles were made by resuspending the mitochondrial preparation in 4 vol. of 30 mmol/l potassium phosphate buffer (pH 7.0). This mixture was sonicated three times for 30 s each at 1 min intervals. The sonicated mitochondria were centrifuged at 8250 g for 10 min to sediment unfragmented mitochondria, and the supernatant was recentrifuged at 80 000 g for 40 min to pellet the mitochondrial particles. The pellet was resuspended in 30 mmol/l potassium phosphate buffer and used for further assay or storage at –80 °C [13,14].

O2− generation monitoring

The rate of O2− generation by submitochondrial particles was measured as described by Boveris [15]. Both the test and reference cuvettes contained 20 to 40 µl of submitochondrial homogenates, 0.1 mol/l potassium phosphate buffer (pH 7.4), 7.2 µmol/l cytochrome c, 0.6 µmol/l antimycin A and 7 mmol/l succinate. SOD (superoxide dismutase; 200 units/ml; Sigma, St Louis, MO, U.S.A.) was added to the reference cuvette. The reduction of cytochrome c was monitored spectrophotometrically at 550 nm. As both the test and reference cuvettes contained identical ingredients, except that the latter included SOD, the measured rate of cytochrome c reduction was specific because of its interaction with O2−.
Measurements of CBF, brain $P_{O_2}$ and $T_b$ (brain temperature)

A 100-µm-diameter thermocouple and two 230 µm fibres 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 Ltd, Oxford, U.K.) is a two-channel device (measuring $P_{O_2}$ and temperature at two sites simultaneously), whereas OxyFlo™ 2000 is a 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 urethane anaesthesia, the animal was placed in a stereotaxic apparatus, and the combined probe was implanted into the corpus striatum using the atlas and coordinates of Paxinos and Watson [5]. The probe calibration parameters were transferred from the probe packaging to the OxyLite™ Instrument using the bar code wand. For each $P_{O_2}$ input on the OxyLite™, there is a corresponding temperature input. A thermocouple may be attached to these temperature inputs using the thermocouple adapters provided. The temperature measurement serves two purposes: (i) to automatically compensate the $P_{O_2}$ measurement, and (ii) to continuously monitor tissue temperature. OxyLite™ is a laser Doppler flow meter whose primary purpose is to measure real-time microvascular red blood cell perfusion. Laser Doppler signals were recorded in BPU (blood perfusion units), which are a relative unit scale defined using a carefully controlled motility standard. The OxyFlo™ is calibrated before leaving the factory using a motility standard solution of carefully selected latex spheres undergoing Brownian motion. The OxyFlo™ is a stable instrument and should not under normal circumstances require recalibration.

Neuronal damage score

At the end of the experiments, animals were killed by an overdose of sodium pentobarbital, and the brains were fixed in situ and left in the skull in 10 % neutral buffered formalin for at least 24 h prior to removal from the skull. The brain was removed and embedded in paraffin blocks. Serial (10 µm) sections through the striatum were stained with haematoxylin/eosin for microscopic evaluation. The extent of cerebral neuronal damage was scored on a scale of 0–3, modified from the grading system of Pulsinelli et al. [16], in which 0 is normal, 1 indicates approx. 3 % of the neurons are damaged, 2 indicates that approx. 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.

### Table I  Effects of heat exposure ($T_a = 42^\circ C$) on the latency for onset of heatstroke, survival time and mortality rate in different groups of rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Latency (min)</th>
<th>Survival time (min)‡</th>
<th>Mortality rate (n)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS-pretreated rats at 42 °C</td>
<td>&gt; 400</td>
<td>&gt; 400</td>
<td>0/8</td>
</tr>
<tr>
<td>NS-pretreated rats at 24 °C</td>
<td>88 ± 2∗</td>
<td>24 ± 3*</td>
<td>8/8</td>
</tr>
<tr>
<td>Albumin-pretreated rats at 42 °C</td>
<td>103 ± 4†</td>
<td>101 ± 16†</td>
<td>8/8</td>
</tr>
<tr>
<td>NS-post-treated rats at 42 °C</td>
<td>87 ± 3*</td>
<td>22 ± 2*</td>
<td>8/8</td>
</tr>
<tr>
<td>Albumin-post-treated rats at 42 °C</td>
<td>105 ± 5†</td>
<td>96 ± 15†</td>
<td>8/8</td>
</tr>
</tbody>
</table>

‡ All groups exposed to 42 °C had heat exposure withdrawn at 88 min.

Statistical analysis

Data are presented as means ± S.E.M. A one-way ANOVA was used for factorial experiments, whereas Duncan’s multiple-range test was used for post-hoc multiple comparison among means. Wilcoxon tests were used for evaluation of neuronal damage scores. Wilcoxon tests convert the scores or values of a variable into 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. A P value less than 0.05 was considered to be statistically significant.

RESULTS

The latency values for the onset of heatstroke and the survival times when rats were exposed to 42 °C for 88 min are shown in Table 1. However, the mortality rates during 88 min of heat exposure plus 392 min room temperature exposure were not different among the groups (Table 1).

The effects of heat exposure (42 °C for 88 min) on several physiological parameters in NS- and albumin-treated rats are shown in Table 2. In NS-treated groups, the values of HR, ICP and $T_b$ were significantly increased at 100 min after the start of heat exposure compared with those of normothermic controls. In contrast, the values of MAP, CPP (= MAP – ICP), CBF and $P_{O_2}$ were significantly lower than those of the normothermic controls. Treatment with an i.v. dose of 10 % albumin immediately after the start of heat exposure or heatstroke onset significantly attenuated the heat-stress-induced arterial hypotension, intracranial hypertension, cerebral hypoperfusion and decreased brain $P_{O_2}$. It should be noted that 10 % albumin decreased Hct from the normal
value of 42 % to 26 % and maintained this new level for at least 90 min (Table 2). The time course changes in these parameters are shown in Figure 1.

The effects of heat exposure (42 °C for 88 min) on the extent of lipid peroxidation and the rate of $O_2^-$ generation in the striatum of the rat brain are shown in Table 3. In the NS-pretreated or NS-post-treated rats killed at 12 min after the onset of heatstroke, the degree of lipid peroxidation and rate of $O_2^-$ generation in the striatum were greater than those of normothermic controls. Again, the heat-stress-induced enhancement in both the extent of lipid peroxidation and rate of $O_2^-$ generation in striatum were attenuated by treatment of rats with 10 % albumin right after the start of heat exposure or at heatstroke onset.

Table 4 shows the effect of heat stress (42 °C for 88 min) on extracellular levels of glutamate, glycerol, lactate/pyruvate ratio and hydroxyl radicals in the striatum of rats. The values of glutamate, glycerol, lactate/pyruvate ratio and hydroxyl radicals in the striatum of heatstroke rats receiving NS immediately after the start of heat exposure or heat stroke onset were significantly greater than those of normothermic controls. However, the values in heatstroke rats receiving 10 % albumin immediately after the start of heat exposure or heatstroke onset were significantly lower than those of the heatstroke rats receiving NS. The time course changes in these parameters are shown in Figure 2.

![Figure 1](image-url) Effects of heat stress on MAP, ICP, CPP, CBF, brain $P_{O_2}$, $T_B$, and Hct in rats pretreated with NS (●) or 10 % albumin (○) at the onset of heat exposure. Injection, time of NS and albumin injection and the start of heat exposure; onset, mean time of heatstroke onset in the NS-pretreated group. * $P < 0.05$ compared with preheated controls; † $P < 0.05$ compared with NS-treated rats (ANOVA). Points represent means ± S.E.M. for eight rats/group.
Table 3  Effects of heat exposure (HE; 42 °C for 88 min) on the extent of lipid peroxidation and rate of O$_2^{**}$ generation in mitochondria in the tissue homogenates of corpus striatum measured at 100 min (i.e. 12 min after heat exposure was withdrawn) in rats treated with NS or albumin immediately before the start of HE or at the onset of heatstroke.

Values are means ± S.E.M. for eight rats per group. *P < 0.05 compared with NS-treated rats at 24 °C (ANOVA, followed by Duncan’s test). †P < 0.05 compared with NS-treated rats at 42 °C (ANOVA, followed by Duncan’s test). RFU, relative fluorescent units.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Lipid peroxidation (RFU)</th>
<th>Rate of O$_2^{**}$ generation (nmol·min$^{-1}$·mg$^{-1}$ of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS-pretreated rats at 24 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min after treating</td>
<td>1.71 ± 0.13</td>
<td>6.72 ± 0.73</td>
</tr>
<tr>
<td>100 min after treating</td>
<td>1.68 ± 0.12</td>
<td>6.65 ± 0.71</td>
</tr>
<tr>
<td>NS-pretreated rats at 42 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min before HE</td>
<td>1.66 ± 0.12</td>
<td>6.85 ± 0.69</td>
</tr>
<tr>
<td>100 min after HE</td>
<td>2.18 ± 0.15*</td>
<td>9.11 ± 0.87*</td>
</tr>
<tr>
<td>Albumin-pretreated rats at 42 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min before HE</td>
<td>1.73 ± 0.14</td>
<td>6.92 ± 0.72</td>
</tr>
<tr>
<td>100 min after HE</td>
<td>1.88 ± 0.11†</td>
<td>7.15 ± 0.72†</td>
</tr>
<tr>
<td>NS-post-treated rats at 42 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min before HE</td>
<td>1.69 ± 0.12</td>
<td>6.74 ± 0.69</td>
</tr>
<tr>
<td>100 min after HE</td>
<td>2.21 ± 0.16*</td>
<td>9.23 ± 0.85*</td>
</tr>
<tr>
<td>Albumin-post-treated rats at 42 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min before HE</td>
<td>1.72 ± 0.13</td>
<td>6.85 ± 0.69</td>
</tr>
<tr>
<td>100 min after HE</td>
<td>1.79 ± 0.10†</td>
<td>7.09 ± 0.71†</td>
</tr>
</tbody>
</table>

Table 4  Effects of heat exposure (HE; 42 °C for 88 min) on mean $T_{co}$, MAP and extracellular concentrations of glutamate, glycerol, lactate/pyruvate and DHBA in the corpus striatum of rats pretreated with NS or albumin immediately before the start of HE or rats post-treated with NS or albumin 12 min after the onset of heatstroke.

Values are means ± S.E.M. for eight rats per group. *P < 0.05 compared with NS-treated rats at 24 °C (ANOVA, followed by Duncan’s test). †P < 0.05 compared with NS-treated rats at 42 °C (ANOVA, followed by Duncan’s test).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$T_{co}$ (°C)</th>
<th>MAP (mmHg)</th>
<th>Glutamate (µmol/l)</th>
<th>Glycerol (µmol/l)</th>
<th>Lactate/pyruvate</th>
<th>DHBA (% baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS-pretreated rats at 24 °C</td>
<td>36.2 ± 0.2</td>
<td>79 ± 5</td>
<td>0.88 ± 0.52</td>
<td>3 ± 1</td>
<td>6 ± 2</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>0 min after treating</td>
<td>36.1 ± 0.3</td>
<td>75 ± 4</td>
<td>0.97 ± 0.63</td>
<td>4 ± 2</td>
<td>7 ± 3</td>
<td>98 ± 4</td>
</tr>
<tr>
<td>100 min after treating</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS-pretreated rats at 42 °C</td>
<td>36.0 ± 0.3</td>
<td>78 ± 4</td>
<td>0.95 ± 0.53</td>
<td>3 ± 2</td>
<td>6 ± 3</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>0 min before HE</td>
<td>42.6 ± 0.2*</td>
<td>2 ± 2*</td>
<td>54 ± 8*</td>
<td>22 ± 4*</td>
<td>127 ± 34*</td>
<td>705 ± 26*</td>
</tr>
<tr>
<td>100 min after HE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS-post-treated rats at 42 °C</td>
<td>36.3 ± 0.3</td>
<td>77 ± 3</td>
<td>0.93 ± 0.48</td>
<td>4 ± 2</td>
<td>7 ± 2</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>0 min before HE</td>
<td>42.8 ± 0.3</td>
<td>50 ± 4†</td>
<td>2 ± 1†</td>
<td>5 ± 2†</td>
<td>8 ± 4†</td>
<td>124 ± 8†</td>
</tr>
<tr>
<td>100 min after HE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin-pretreated rats at 42 °C</td>
<td>36.1 ± 0.2</td>
<td>81 ± 4</td>
<td>0.85 ± 0.53</td>
<td>4 ± 2</td>
<td>7 ± 3</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>0 min before HE</td>
<td>42.7 ± 0.3*</td>
<td>4 ± 3*</td>
<td>51 ± 7*</td>
<td>24 ± 3*</td>
<td>124 ± 32*</td>
<td>694 ± 22*</td>
</tr>
<tr>
<td>100 min after HE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS-post-treated rats at 42 °C</td>
<td>36.2 ± 0.2</td>
<td>79 ± 4</td>
<td>0.91 ± 0.49</td>
<td>3 ± 2</td>
<td>6 ± 2</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>0 min before HE</td>
<td>42.5 ± 0.3</td>
<td>52 ± 5†</td>
<td>3 ± 2†</td>
<td>4 ± 2†</td>
<td>7 ± 3†</td>
<td>119 ± 7†</td>
</tr>
<tr>
<td>100 min after HE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The effects of heat exposure (42 °C for 88 min) on neuronal damage in striatum of rats pretreated or post-treated with NS or albumin were determined. Again, it was found that the scores for striatal neuronal damage in heatstroke rats receiving NS immediately after the start of heat exposure [median (Q1, Q3), 2 (2, 2)] or at heatstroke onset [median (Q1, Q3), 2 (2, 2)] were significantly greater ($P < 0.05$) than those of the normothermic controls [median (Q1, Q3), 0 (0, 0.75)]. However, the striatal neuronal damage scores in heatstroke rats receiving 10% albumin immediately after the start of heat exposure [median (Q1,Q3), 0 (0, 1)] or at heatstroke onset [median
Albumin therapy attenuates heatstroke

Figure 2  Effects of heat stress on $T_{co}$, MAP and the extracellular concentrations of glutamate, glycerol and lactate/pyruvate ratio in the corpus striatum of rats pretreated with NS (□) or 10 % albumin (●) at the onset of heat exposure.

Injection, time of NS and albumin injection and the start of heat exposure; onset, mean time of heatstroke onset in the NS-pretreated group. Values are means ± S.E.M. for eight rats/group. *P < 0.05 compared with preheated controls; +P < 0.05 compared with NS-treated rats (ANOVA).

(Q1,Q3), 0 (0,1)] were significantly lower (P < 0.05) than those of the heatstroke rats receiving NS controls.

**DISCUSSION**

The lactate/pyruvate ratio is a well-known marker of cellular ischaemia, whereas glycerol is a marker of how severely cells are affected by ongoing pathology [17–20]. Excessive accumulation of glutamate has been shown in ischaemic brain tissue [21,22]. Indeed, as shown in the present results, cerebral ischaemia induced by heatstroke is associated with an increased production of glycerol, lactate/pyruvate ratio and glutamate in the brain. In addition, $P_{O_2}$ in the striatum of rat brain was decreased after the onset of heatstroke. Therefore, after the onset of heatstroke, the excessive accumulation of glycerol, glutamate and lactate/pyruvate ratio in the brain may be secondary to cerebral ischaemia and injury in rats. The present results showed further that intravenous infusion of 10 % human albumin at the start of heat exposure or heatstroke onset, in addition to reducing blood Hct from the control value of 42 % to 26 % for at least 90 min, significantly attenuated the increased cerebral levels of glycerol, glutamate and lactate/pyruvate ratio as well as preventing the ischaemia and brain injury induced by heatstroke in rats. Accordingly, hypervolaemic haemodilution prolonged survival in heatstroke rats.

Hypervolaemic haemodilution has been used to treat animals with acute cerebral ischaemia by increasing blood flow through a reduction in the viscosity and $O_2$ content of the blood, caused by reduction in both Hct and fibrinogen [23–25]. As demonstrated in the present results, the increased survival in heatstroke rats treated with hypervolaemic haemodilution was associated with augmentation of MAP, local CBF and oxygenation, as well as a reduction in both ICP and cerebral neuronal injury associated with heatstroke. The augmentation of CBF and oxygenation in animals receiving 10 % albumin was brought about by higher CPP resulting from lower ICP (this may be due to a reduction in cerebral oedema and cerebro-ventricular congestion) and higher MAP during the development of heatstroke [26–28]. The present results are, in part, consistent with several previous findings showing that other hypertonic agents (such as hypertonic saline and mannitol) produce both volume expansion and maintenance of appropriate MAP in patients with haemorrhagic shock [29]. The resulting hypervolaemic haemodilution may then reduce the circulatory shock by increasing cardiac output (due to an increase in ventricular depolarization or stroke volume) [30].

Recently, we have shown [1] that heatstroke-induced cerebral ischaemia and neuronal damage is associated with an increased production of free radicals (specifically hydroxyl radical and $O_2^−$), higher lipid peroxidation and lower enzymic antioxidant defences in rats. Again, as demonstrated in the present results, hypervolaemic haemodilution significantly attenuated the increased levels of free radicals and lipid peroxidation in the brain associated with heatstroke. Thus it is evident that heatstroke-induced oxidative stress in the brain can be reduced by hypervolaemic haemodilution.

It has been reported [31] that within the first few days after a stroke, intracranial hypertension, brain tissue shifts and tentorial herniation are the main causes of death. In this context, hypertonic solutions have been prescribed for treatment of intracranial hypertension. Various clinical and experimental studies have shown that a single dose of mannitol was also able to reduce elevated intracranial hypertension [29,32]; however, mannitol failed to be effective in some patients with head injuries, especially after repeated doses. Repeated mannitol therapy may even aggravate brain oedema [33,34]. In addition, evidence has accumulated to demonstrate that hypertonic saline may be an effective therapy for both brain oedema and intracranial hypertension after head
trauma \[35–37\]. Bolus infusion of hypertonic saline led to a reduction in intracranial hypertension in all patients with elevated ICP after acute space-occupying hemispheric stroke or supratentorial haemorrhage with massive perifocal oedema, whereas previous treatment with mannitol was ineffective \[30\]. However, repeated use of hypertonic saline will be limited by hypernatraemia \[16\]. Bolus infusion of hypertonic saline will be limited by hypernatraemia \[16\].

In summary, the present study has shown that 10 % human albumin attenuated heatstroke-induced arterial hypotension, intracranial hypertension, cerebral hypoperfusion and the elevation in the striatal levels of glutamate, glycerol, lactate and free radicals. Albumin therapy greatly decreased cerebral neuronal damage and resulted in a prolongation of survival time. However, 10 % human albumin therapy did not influence mortality rates with the duration of heat exposure used in the current experiment (88 min heat exposure at 42 °C, followed by 392 min at 24 °C). These results suggest that 10 % human albumin does not prevent the heatstroke syndromes entirely but does attenuate them.

ACKNOWLEDGMENTS

Financial support for this study was provided by grants from both the National Science Council of Republic of China (NSC 92-2314-B-195-017) and Chi-Mei Foundation Hospital.

REFERENCES


Received 7 August 2003/16 December 2003; accepted 19 December 2003
Published as Immediate Publication 19 December 2003, DOI 10.1042/CS20030263