Hypothalamic Dopamine Release and Local Cerebral Blood Flow During Onset of Heatstroke in Rats

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Background and Purpose Brain dopamine has been implicated as a mediator of brain neuronal damage resulting from ischemic injury. Augmented interleukin-1 production and cerebral ischemia occurred during onset of heatstroke. This study has attempted to ascertain whether heatstroke resulting from hyperthermia causes an increase in hypothalamic dopamine release and to assess whether the administration of an interleukin-1 receptor antagonist (IL-1ra) can attenuate heatstroke formation.

Methods Both local cerebral blood flow and hypothalamic dopamine release during onset of heatstroke were assessed in saline-treated rats and in rats treated with an IL-1ra. Heatstroke was induced by exposing the animals to a high ambient temperature. Hypothalamic dopamine release was determined by carbon fiber electrodes combined with in vivo differential pulse amperometry.

Results During onset of heatstroke, rats with heatstroke displayed higher values of colonic temperature, higher values of hypothalamic dopamine release, and lower values of blood flow in different brain regions compared with normothermic control rats. In another separate experiment in which IL-1ra (200 µg/kg IV) was injected 30 minutes before onset of heatstroke, both the augmented hypothalamic dopamine release and diminished cerebral blood flow during onset of heatstroke were significantly attenuated. In addition, the survival time (interval between onset of heatstroke and death) of the rats with heatstroke was prolonged by pretreatment with IL-1ra.

Conclusions These results suggest that an increase in hypothalamic dopamine release and a decrease in local cerebral blood flow occur during onset of heatstroke. Pretreatment with IL-1ra attenuates the heatstroke formation resulting from cerebral ischemia by reducing hypothalamic dopamine release.

Key Words • heatstroke • interleukin-1 receptor antagonist • cerebral blood flow • dopamine

Subjects and Methods

Materials IL-1ra, used in the present study, was a gift from Dr. James L. Vannice (SYNERGEN). This protein was expressed in Escherichia coli using a cDNA originally isolated from adherent monocytes. IL-1ra is the nonglycosylated, N-terminal methionyl form of the naturally occurring protein and has a molecular weight of approximately 17 kD. IL-1ra blocks binding of IL-1 as well as the naturally occurring, glycosylated form does.

Animal Surgery and Measurement of Physiological Parameters The right femoral artery and vein of adult male Sprague-Dawley rats (weighing between 250 and 300 g), under urethane (1.4 g/kg IP) anesthesia, were cannulated with polyethylene tubing (PE 50). Systemic arterial blood pressure was monitored continuously with a pressure transducer and a chart recorder (Gould model 481). The animals were then fixed to a stereotaxic frame. Colonic temperature was monitored continuously by thermocouples. The levels of extracellular dopamine-like materials in the right hypothalamus were recorded continuously with a Biopulse voltammetry (Solea Tacussel Co), as we previously reported. In brief, a carbon fiber electrode was used as a working electrode to measure the oxidation current of dopamine and was inserted into the right hypothalamus using the coordinates of A, 7.0 mm; L, 0.8 mm; H, −1.0 mm. After conclusion of the experiments, 4-mA anodal DC was passed through the working electrodes for 10 seconds. The lesions induced in this way were used to identify the actual electrode
Treatment | Time Course | Colonic Temperature, °C | Mean Arterial Pressure, mm Hg | DA, % baseline | Survival Time, min
--- | --- | --- | --- | --- | ---
0.9% Saline IV | 1. 30 min before HS | 38.6±0.36 | 100.5±4.4 | 118±5 | 
2. At onset of HS | 42.3±0.24* | 85.5±7.1* | 156±6* | 
3. Just before death | 42.3±0.15* | 47.3±3.7* | 274±12* | 15±5 |
IL-1ra, 200 µg/kg IV | 1. 30 min before HS | 39.0±0.5 | 109.0±9.0 | 108±7 | 
2. At onset of HS | 42.6±0.11 | 121.3±4.3† | 103±20† | 
3. Just before death | 36.4±0.85† | 48.7±4.7 | 88±16† | 200±8† |

IL-1ra indicates interleukin-1 receptor antagonist; HS, heatstroke; and DA, dopamine.

*P<.05, significantly different from control values (30 minutes before HS; saline group), ANOVA.
†P<.05, significantly different from control values (saline group), ANOVA.

Treatment | Local Cerebral Blood Flow, mL · 100g⁻¹ · min⁻¹
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RCx | LCx | RBGL | LBGL | RTH | LTH
Normotherm control rats,
0.9% saline IV | 160.9±11.4 | 158.4±9.5 | 143.2±6.6 | 137.5±11.4 | 153.2±9.0 | 154.4±11.5
Heatstroke rats,
0.9% saline IV | 106.7±7.6* | 97.3±7.6* | 98.6±9.1* | 92.6±5.3* | 96.2±2.4* | 97.9±3.7*
IL-1ra, 200 µg/kg IV | 200.3±13.0† | 191.1±24.8† | 196.9±30.6† | 186.9±28.3† | 132.6±8.0† | 135.6±8.0†

RCx indicates right cortex; LCx, left cortex; RBGL, right basal ganglia; LBGL, left basal ganglia; RTH, right thalamus; LTH, left thalamus; RFrWM, right frontal white matter; LFrWM, left frontal white matter; RHTH, right hypothalamus; LHTH, left hypothalamus; and IL-1ra, interleukin-1 receptor antagonist.

Values are mean±SEM of four rats.
*P<.05, significantly different from control values (normothermia control rats), ANOVA.
†P<.05, significantly different from control values (heatstroke, saline group), ANOVA.

The procedure for measuring local cerebral blood flow was similar to those used previously. In rats, approximately 50 µCi of iodo[14C]antipyrine in 1 mL of normal saline was infused at a constant rate through the femoral venous catheter for 1 minute, during which time arterial samples were collected on filter paper disks for assay of arterial concentration. At exactly 1 minute, the animal was decapitated, and the brain was removed, frozen, and assayed for 14C concentration by the autoradiographic technique. Autoradiographs of sections of rat brain and calibrated plastic 14C standards were used to determine tissue concentrations of 14C by densitometric measurements.

Measurement of Local Cerebral Blood Flow

The rats, under urethane anesthesia, to an ambient temperature of 42°C. The movement in which mean arterial blood pressure began to decrease from its peak level was taken as onset of heatstroke, as shown in the Figure. The animals were given an intravenous dose of 1 mL/kg of 0.9% saline per 1 kg body weight 30 minutes before the onset of heatstroke. Rats with heatstroke that received IL-1ra treatment: the rats, under urethane anesthesia, were given an intravenous dose of 200 mg/mL of IL-1ra per 1 kg body weight 30 minutes before onset of heatstroke. (3) Normothermic control rats: the rats, under urethane anesthesia, were exposed to an ambient temperature of 24°C for at least 90 minutes. Then they were given an intravenous dose of 1 mL/kg of 0.9% saline 30 minutes before death for cerebral blood flow assay. Their colonic temperatures were kept at about 37°C by an electric thermal mat.

Results

Table 1 summarizes the mean and SEM values for each measurement of the various parameters collected from two groups of animals. Heatstroke was induced by exposing the animals to an ambient temperature of 42°C. The latency of onset of heatstroke was found to be 60 minutes for all these animals. As Table 1 shows, rats that received saline injection 30 minutes before onset of heatstroke displayed higher values of colonic temperature, lower values of mean arterial blood pressure, and higher values of dopamine release in the hypothalamus compared with their preinjection controls. Table 1 also shows that pretreatment of rats with an intravenous dose of IL-1ra (200 µg/kg) 30 minutes before onset of heatstroke significantly prolonged survival time (inter-
Graphs show time course changes of colonic temperature (Tco), mean arterial pressure (MPa), and hypothalamic dopamine release produced by a high ambient temperature (Ta) in rats treated with saline and with IL-1ra. Onset of heatstroke occurred at time 0, and saline or IL-1ra was injected 30 minutes before onset of heatstroke.

Table 2 summarizes the mean and SEM values for blood flow in 10 structural components of the normothermic control rat brain obtained with iodo\(^{14C}\)antipyrine and compares them with the values obtained in paired animals during onset of heatstroke. The values obtained from animals suffering from heatstroke were significantly lower than those obtained from normothermic control rats in all structures. The table also shows that the reduced local cerebral blood flow values in all structural components of the brains from rats with heatstroke were restored to normal levels by pretreatment of animals with IL-1ra 30 minutes before onset of heatstroke.

**Discussion**

Our previous results\(^2\)–\(^4\) demonstrated that during onset of heatstroke animals displayed hyperthermia, systemic hypotension, intracranial hypertension, decreased cerebral perfusion, cerebral ischemia, and degeneration of neurons in different brain regions, including the hypothalamus and corpus striatum. During the fever plateau, however, the febrile animals did not show the above-mentioned physiological dysfunction and brain neuronal loss, although they had similar levels of hyperthermia.\(^3\),\(^5\) These observations suggested that cerebral ischemia or anoxia was the main cause for development of heatstroke syndromes (in particular, the central nervous system dysfunctions). The present results, obtained with the in vivo voltametry, demonstrated that during onset of heatstroke a massive amount of dopamine was released into the extracellular fluid compartment of rat hypothalamus, and blood flow in the hypothalamus and other brain regions was reduced significantly. In fact, it has been concluded that dopaminergic hyperactivity is necessary for cell death in brain during cerebral ischemia or anoxia.\(^14\) The ischemic damage to brain neurons was reduced by depleting the brain dopamine concentration with intracerebroventricular injection of 6-hydroxydopamine.\(^15\) As Graham\(^16\) suggested, sustained auto-oxidation of dopamine could lead to excessive accumulation of toxic quinones and potentially cytotoxic oxygen free radicals. These observations prompted us to think that the dopaminergic hyperactivity in the brain is responsible for the selective vulnerability of brain neurons to cerebral ischemia during onset of heatstroke. The dopaminergic hyperactivity was proposed to contribute to selective vulnerability of cerebral neurons to ischemic injury either by accentuation of postischemic hypoperfusion or by a direct metabolic effect.\(^5\)

Some experimental evidence suggests that pyrogens of intestinal origin play a role in heatstroke formation. At least in experimental animals, immunization against bacterial endotoxin\(^17\) or administration of antibiotics before heating sharply reduces mortality.\(^18\) Our recent results\(^8\) also showed that during onset of heatstroke rabbits displayed augmented IL-1\(\beta\) production (in both the plasma and the hypothalamus) compared with that of normothermic control rabbits. In addition, the present results showed that the animals with heatstroke that received an intravenous injection of IL-1ra had a survival time longer than the animals with heatstroke that received control-vehicle solution. Could the fact that...
the IL-1ra merely prolonged survival time but did not reduce mortality be a result of the short half-life of IL-1ra? Would additional injections of IL-1ra reduce mortality? These questions must be answered by further studies in the near future. Furthermore, in our experiments in which IL-1ra was intravenously injected 30 minutes before onset of heatstroke, both the extracellular dopamine levels in the hypothalamus and local cerebral blood flow were restored to control levels. These results suggest that IL-1ra attenuates heatstroke formation resulting from cerebral ischemia or anoxia by reducing the hypothalamic extracellular dopamine release in rats. However, the attenuation of heatstroke by IL-1ra could be caused by some effect other than a reduction in dopamine release. For example, the brain temperature in the control animals where dopamine is the highest is 42.3°C, whereas in the IL-1ra–treated animals the brain temperature just before death was 36.4°C. This substantial difference in brain temperature could have been the cause of the reduced release of dopamine. It should be mentioned that other interleukins, such as TNF-α and IL-6, may play a role in heatstroke.

In a recent study, in vivo voltammetry was used in rats with transient cerebral ischemia by temporary clamping of the right common carotid artery, systemic hypotension, and intracranial hypertension to measure changes in extracellular dopamine concentrations in the corpus striatum. Striatal neuronal damage was also rated on a scale of 0 to 3 (0, no damage; 3, maximum cell loss). It was found that striatal dopamine levels increased by 12-fold and that striatal neuronal damage rated 2.5 during 30 minutes of brain ischemia. The dopamine levels returned to control levels 30 minutes after reperfusion. In a separate experiment in which 10% human albumin was intravenously infused 30 minutes before brain ischemia, both striatal dopamine levels and striatal neuronal damage were reduced compared with ischemic controls. These results suggest that hypervolemic hemodilution protects the striatal neurons from ischemic injury by reducing the extracellular dopamine release in the corpus striatum of rat brain. Hypervolemic hemodilution may have augmented cerebral blood flow by decreasing blood viscosity and rheological changes and resulted in reduction of dopamine release. Therefore, we hypothesize that hypervolemic hemodilution may also protect against heatstroke formation from ischemic injury by reducing the extracellular dopamine release in the brain. Of course, this possibility needs to be verified by further studies.

References

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Editorial Comment

Although increases in interleukin-1 (IL-1) levels have been associated with brain injury, the evidence for a role of IL-1 in potentiating damage is meager. Preliminary studies with an IL-1 receptor antagonist have suggested that blockade of IL-1 receptors can reduce ischemic brain edema and excitatory amino acid-induced neurotoxicity. In the preceding study by Kao et al, intravenous administration of an IL-1 antagonist 30...
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