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Aminoguanidine Protects Against Intracranial Hypertension and Cerebral Ischemic Injury in Experimental Heatstroke

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Abstract. The aim of the present study was to ascertain whether aminoguanidine attenuated intracranial hypertension and cerebral ischemic injury in experimental heatstroke. Urethane-anesthetized rats were exposed to heat stress (ambient temperature of 43°C) to induce heatstroke. Control rats were exposed to 24°C. Mean arterial pressure, cerebral perfusion pressure, and cerebral blood flow after the onset of heatstroke were all significantly lower than in control rats. However, colonic temperature, intracranial pressure, heart rate, cerebral inducible nitric oxide synthase (iNOS)-dependent NO, and neuronal damage score were greater after the onset of heatstroke. Aminoguanidine (30 μmol/kg, i.v.; 30 min before the start of heat exposure) pretreatment significantly attenuated the heatstroke-induced hyperthermia, arterial hypotension, intracranial hypertension, cerebral ischemia and neuronal damage, and increased iNOS-dependent NO formation in the brain. The extracellular concentrations of ischemic (e.g., glutamate and lactate/pyruvate ratio) and damage (e.g., glycerol) markers in the hypothalamus were also increased after the onset of heatstroke. Aminoguanidine pretreatment significantly attenuated the increase in hypothalamic ischemia and damage markers associated with heatstroke. Delaying onset of aminoguanidine administration (i.e., 0 or 30 min after the start of heat exposure) reduced the preventive efficiency on heatstroke-induced hyperthermia, arterial hypotension, intracranial hypertension, cerebral ischemia, and increased iNOS-dependent NO formation in brain. These results suggest that aminoguanidine protects against heatstroke-induced intracranial hypertension and cerebral ischemic injury by inhibition of cerebral iNOS-dependent NO production.

Keywords: aminoguanidine, heatstroke, nitric oxide, cerebral ischemia, hypothalamus

Introduction

In rats, heat stress leads to increased metabolic demand and reduced splanchnic blood flow, which in turn induce intestinal and hepatocellular hypoxia; the hypoxia results in the generation of highly reactive oxygen and nitrogen species that accelerate mucosal injury (1, 2). Intestinal mucosal permeability to endotoxin increases in heat-stressed rats (3). This alteration allows leakage of endotoxins and increases production of inflammatory cytokines that induce release of nitric oxide (NO) and endothelins. Both pyrogenic cytokines and endothelium-derived factors can interfere with normal thermoregulation, thereby precipitating arterial hypotension, hyperthermia, and heatstroke (4). Plasma or brain nitrite/nitrate levels are elevated in heatstroke patients (5) and rats (6, 7). A more recent report has also shown that aminoguanidine (AG) slows the rate of NO production, preserves the splanchnic blood flow, and improves heat tolerance in rats (2).

In addition, the heat-stressed rats, in addition to arterial hypotension, display intracranial hypertension, brain hypo-perfusion, and cerebral ischemia and injury (4, 8). Aminoguanidine might protect against the intracranial hypertension and cerebral ischemic injury in heatstroke rats.

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Therefore, in order to validate the matter, the present experiments were performed to assess the effects of heat stress on intracranial pressure (ICP) values, local cerebral blood flow (CBF) values, neuronal damage scores, and the extent of inducible nitric oxide synthase (iNOS)-dependent NO in rat brain with or without prior administration of AG (an iNOS inhibitor) (9). In addition, we assessed the preventive effect of AG on the extracellular levels of cerebral ischemia and injury markers associated with heat stress.

Materials and Methods

Experimental groups

Adult male Sprague-Dawley rats weighing 300 and 350 g were obtained from the Animal Resource Center of the Chi-Mei Medical Center (Tainan, Taiwan). Between experiments, the animals were housed in group cages at an ambient temperature (Ta) of 24 ± 1°C with a 12-h light/dark cycle, with the lights being switched on at 6:00 AM. Animal chow and water were allowed ad libitum.

Animals were assigned randomly to one of the following three major groups. One group of rats, treated with 0.9% NaCl solution per ml per kilogram of body weight 30 min before the start of heat exposure, was exposed to Ta of 43°C (with relative humidity of 60% in a temperature-controlled chamber). At a certain point in this heatstroke group, local CBF in the hypothalamus of rat brains began to decrease from their peak levels; this instant was considered as the onset of heatstroke (10). Immediately after the onset of heatstroke, heat stress was terminated and the Ta was restored to 24°C. Our results showed that the latency for the onset of heatstroke (i.e., the interval between the start of heat exposure and the onset of heatstroke) were 63 ± 3 (n = 8) min for rats treated with intravenous injections of 0.9% NaCl solution 30 min before the start of heat exposure. The second group of animals was treated with an intravenous dose of 30 μmol/kg of AG (Sigma Chemical Co., St. Louis, MO, USA) 30 min before the start of heat exposure. We chose a concentration of 30 μmol/kg of AG based on the work of Grisham et al. (11) and Corbett et al. (12) who demonstrated in vivo the relative specificity of AG for iNOS over eNOS and nNOS. These two experimental groups of rats were exposed to Ta of 43°C for 63 min. Then the Ta was restored to 24°C and the survival time (interval between the onset of heatstroke and cardiac arrest) of these rats treated with either 0.9% NaCl solution or AG were recorded. The third group of animals was exposed to room temperature (24°C) for at least 90 min to reach thermal equilibrium before the start of experimentation and was used as the normothermic control group. Each group of animals was subjected to the following procedures: a) measurement of colonic temperature (Tco), mean arterial pressure (MAP), CBF, ICP, and cerebral perfusion pressure (CPP = MAP – ICP); b) measurement of extracellular concentrations of glutamate, glycerol, lactate/pyruvate ratio, and NO metabolites (NO_x^-) in the hypothalamus; and c) determination of iNOS immunoreactivity and neuronal damage in the hypothalamus.

All experiments were approved by the Animal Research Committee of the Chi-Mei Medical Center. Adequate anesthesia was maintained to abolish the corneal reflex and pain reflexes induced by tail pinch throughout the course of all experiments (about 4 h) following a single dose of urethane (1.4 g per kg body weight, Sigma Chemical Co.).

Physiological parameter monitoring

The right femoral artery and vein of rats, under urethane (1.4 g/kg intraperitoneally) were cannulated with polyethylene tubing (PE 50) for blood pressure monitoring and drug administration. Tco was monitored continuously by a thermocouple, while both MAP and heart rate were continuously monitored with a pressure transducer. Animals were positioned in a stereotaxic apparatus (model 1460; David Kopf Instrument, Tujunga, CA, USA) to insert probes for the measurement of ICP. The ICP was monitored with a Statham P23AC transducer (Gould Instruments, Cleveland, OH, USA) via a 20-gauge stainless-steel needle probe (diameter, 0.90 mm; length, 38 mm), which was introduced into the right lateral cerebral ventricle according to the stereotaxic coordinates of Paxinos and Watson (13): P, –0.8 mm; L, 1.4 mm; and H, 3.6 mm.

CBF monitoring

Animals, under urethane (1.4 g/kg, i.p.), were positioned in a stereotaxic apparatus (model 1460) to insert a probe for measurement of local CBF in the hypothalamus. A 24-gauge stainless-steel needle probe (diameter, 0.58 mm; length, 40 mm) was inserted into the right hypothalamus using the coordinates of Paxinos and Watson (13): P, –1.8 mm; L, 0.8 mm; and H, 8.8 mm. Local CBF in the hypothalamus was monitored with a Laserflo BPM2 laser Doppler flowmeter (Vasametics, St. Paul, NM, USA).

Extracellular NO monitoring

A microdialysis probe (CMA 20; Carnegie Medicine, Stockholm, Sweden) with a 4-mm-long dialysis membrane was vertically implanted into the left hypothalamus. A Ringer's solution (0.860 g NaCl, 0.030 g

KCl, 0.033 g $CaCl_2$ per 100 ml) was perfused through the microdialysis probe at a constant flow (2.0 μL/min). After 6 h of stabilization, the dialysates from the hypothalamus were collected at 20-min intervals. The NO_x^- concentrations in the dialysates were measured with the Eicom ENO-20 NO_x^- analysis system (Eicom, Kyoto) (14). In the Eicom ENO-20 NO_x^- analysis system, after the NO_2^- and NO_3^- in the sample have been separated by the column, the NO_2^- reacts in the acidic solution with the primary aromatic amine to produce an azo compound. Following this, the addition of aromatic amines to the azo compound results in a coupling that produces a diazo compound and the absorbance rate of the red color in this compound is then measured. The system is capable of detecting up to 0.1 pmol. At that time, experiments were carried out to determine the effects of heatstroke on the NO_x^- release in the hypothalamus. After histological verification of the probe's path, all the data obtained were included in our results.

Measurement of cellular ischemia and injury markers

A microdialysis probe (4 mm in length) (CMA 12, Carnegie Medicine) was stereotaxically placed into the hypothalamus according to the atlas and coordinates of Paxinos and Watson (13). According to the methods described previously (15, 16), an equilibration period of 2 h without sampling was allowed after probe implantation. The microdialysis was perfused at 2.0 μL/min, and the dialysates were sampled in microvials. The dialysates were collected every 10 min in a CMA 40 (Carnegie Medicine) fraction Collector. An 5-μL aliquot of dialysate was injected onto a CMA 600 microdialysis analyzer (Carnegie Medicine) for measurement of lactate, glycerol, pyruvate, and glutamate. Four analysates can be analyzed per sample, and the result is displayed graphically within minutes. The thermal experiments were started after showing stabilization in four consecutive samples. In the present results, an equilibrium period of 2 – 3 h assured a stable level of extracellular substance tested.

Immunohistochemical staining

Rats were killed with intravenous urethane (2.8 g/kg) and were transcardially perfused with heparinized 0.05 mol/L phosphate-buffered saline (PBS) followed by ice-cold 15% sucrose in PBS. The brains were rapidly removed and frozen in liquid nitrogen. Coronal brain sections (5-μm-thick) were cut on a cryostat and were thaw-mounted on gelatin-coated slides.

The endogenous peroxidase was blocked with 10% MeOH / 3% H_2O_2 / sodium phosphate buffer solution mixture (1.8 mM $NaH_2PO_4 \cdot H_2O$, 8 mM $Na_2HPO_4 \cdot H_2O$, 0.15 M NaOH, 2.7 mM KCl) for 30 min at room temperature. Preincubation with 2% normal goat serum (NGS) containing 0.2% Triton X-100 (Singma Chemical Co.) was carried out at room temperature for 1 – 2 h to block non-specific binding of immunoglobin G (IgG). Sections were incubated with the commercially available rabbit anti NOS antiserum (1:50) diluted in 0.2% Triton X-100, 1% NGS, and 0.1% azide (Sigma)/PBS at 4°C overnight, then rinsed with PBS for 30 min, and incubated in biotinylated goat antirabbit IgG (1:500) for 1 – 2 h. After several rinses with PBS, sections were incubated in AB mixture (avidin-biotin complex, 1:200; Vectastain) for 1 – 2 h, incubated in 3-3'-diaminobenzidine (DAB; Daco Co., Copenhagen, Denmark) and nickel ammonium sulfate in the presence of 0.003% H_2O_2, and then mounted on gelatinized slides. The specificity of each antiserum was demonstrated by the absence of stain when diluted primary antiserum was preabsorbed with the respective antigen or was replaced by normal serum. For the negative control, sections were incubated with heat-denatured primary antibody. The results of these immunocytochemistry controls were consistently negative.

Neuronal damage score

At the end of the experiment, the brain was removed, fixed in 10% neutral buffered formalin and embedded in paraffin blocks. Serial (5 μm) sections through the hypothalamus were stained with hematoxylin and 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. (17), in which 0 is normal, 1 indicates that approximately 30% of the neurons are damaged, 2 indicates that approximately 60% of the neurons are damaged, and 3 indicates that approximately 100% of the neurons are damaged. Each hemisphere was evaluated independently without the examiner knowing the experimental conditions. Only those areas not invaded by probes were assessed. Ischemic damage was considered to have occurred in any neurons showing pyknosis of the nucleus and cell shrinkage.

Statistical analyses

Data are presented as means ± S.E.M. Comparisons between groups were performed by one-way analysis of variance (ANOVA) for data in Table 1, Fig. 1, and Fig. 2. Post-hoc comparisons were performed by Duncan's test. For the Table 2 data, Wilcoxon signed rank test was used when only two groups were compared. The 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

Table 1. Effects of heat exposure (HE, Ta = 43°C) on both latency for onset of heatstroke and survival time in saline-treated rats and AG-treated rats

Treatments	Latency (min)	Survival time (min)
Normal saline (ml/kg, i.v.)-treated rats at 24°C	450 ± 18	450 ± 21
Normal saline (ml/kg, i.v.; 30 min before HE)-treated rats at 43°C	63 ± 3*	17 ± 2*
AG (30 μmol/kg, i.v.; 30 min before HE)-treated rats at 43°C	78 ± 4$^+$	150 ± 22$^+$
AG (30 μmol/kg, i.v.; 0 min before HE)-treated rats at 43°C	71 ± 2$^+$	66 ± 4$^+$
AG (30 μmol/kg, i.v.; 30 min after HE)-treated rats at 43°C	65 ± 2	19 ± 2

Data are the means ± S.E.M. of eight rats per group. *P<0.05, significantly different from the corresponding control value (normal saline at 24°C) (ANOVA followed by Duncan's test). ^+P<0.05, significantly different from the corresponding control value (normal saline at 43°C) (ANOVA followed by Duncan's test).

sum necessary to test the null hypothesis at a given significant level. The data were treated by "median", followed by first and third quartile. A P value less than 0.05 was considered as statistical significance.

Results

Effect of heat exposure on both latency for onset of heatstroke and survival time

Table 1 summarizes the effects of heat exposure (43°C Ta) on both the latency for onset of heatstroke and survival time in rats. It can be seen from the table that the latency as well as survival time was found to be 63 ± 3 min (n = 8) or 17 ± 2 min (n = 8), respectively, for rats treated with 0.9% NaCl solution. Both the latency and survival time were significantly increased by an intravenous dose of 30 μmol/kg of AG 30 min before the start of heat exposure.

Effect of heat exposure on Tco, MAP, ICP, CPP, and hypothalamic levels of CBF, glutamate, glycerol, and NO_x^-

Both Figs. 1 and 2 show the effects of heat exposure (43°C for 63 min) on Tco, MAP, ICP, and CPP and hypothalamic levels of CBF, glutamate, glycerol, lactate/pyruvate, and NO_x^- in rats pretreated with 0.9% NaCl solution or AG 30 min before the start of heat exposure. As shown in these two figures, 12 min after the onset of heatstroke in the NaCl-treated group, all the MAP, CPP, and CBF values were significantly lower than those of the normothermic controls (P<0.05). On the other hand, the values of Tco, ICP, and levels of glutamate, glycerol, lactate/pyruvate, and NO_x^- in the extracellular fluids of the hypothalamus of the NaCl-treated group were significantly higher in rats 12 min after the onset of heatstroke than in those of the normothermic controls (P<0.05). Heatstroke-induced hyperthermia, arterial hypotension, intracranial hypertension, cerebral hypoperfusion, cerebral ischemia, and increased levels of glutamate, glycerol, lactate/pyruvate, and NO_x^- in the extracellular fluids of hypothalamus were significantly attenuated by treatment with AG 30 min before the start of heat exposure. Delaying onset of AG injection (i.e., 0 or 30 min after the onset of heat exposure), again, reduced the preventive efficiency on heatstroke-induced hyperthermia, arterial hypotension, intracranial hypertension, cerebral ischemia, and increased levels of NO_x^-, glutamate, glycerol, and lactate/pyruvate in brain.

Effects of heat exposure on both iNOS immunoreactivity and neuronal damage score in hypothalamus

Table 2 summarizes the effects of heat exposure on both the iNOS immunoreactivity and the neuronal damage score values of the hypothalamus in rats treated with normal saline or AG 30 min before the start of heat exposure. In normal saline-treated rats killed 12 min after the onset of heatstroke, both the iNOS immunoreactivity and neuronal damage score values of the hypothalamus were greater than those in the normothermic controls. However, the heatstroke-induced increases of both parameters observed in the hypothalamus were greatly attenuated by AG treatment. A typical example for iNOS immunoreactivity or neuronal damage is, respectively, depicted in Fig. 3 or Fig. 4. Again, delaying onset of AG treatment (i.e., 0 or 30 min after the start of heat exposure) reduced the heatstroke-induced neuronal damage and increased immunoreactivity of iNOS in the brain.

Discussion

This is the first report on the preventive effect of systemic administration of AG on heatstroke. AG pretreatment significantly attenuated the heatstroke-induced hyperthermia, arterial hypotension, intracranial hypertension, cerebral ischemia and neuronal damage, and increased iNOS-dependent NO formation in the

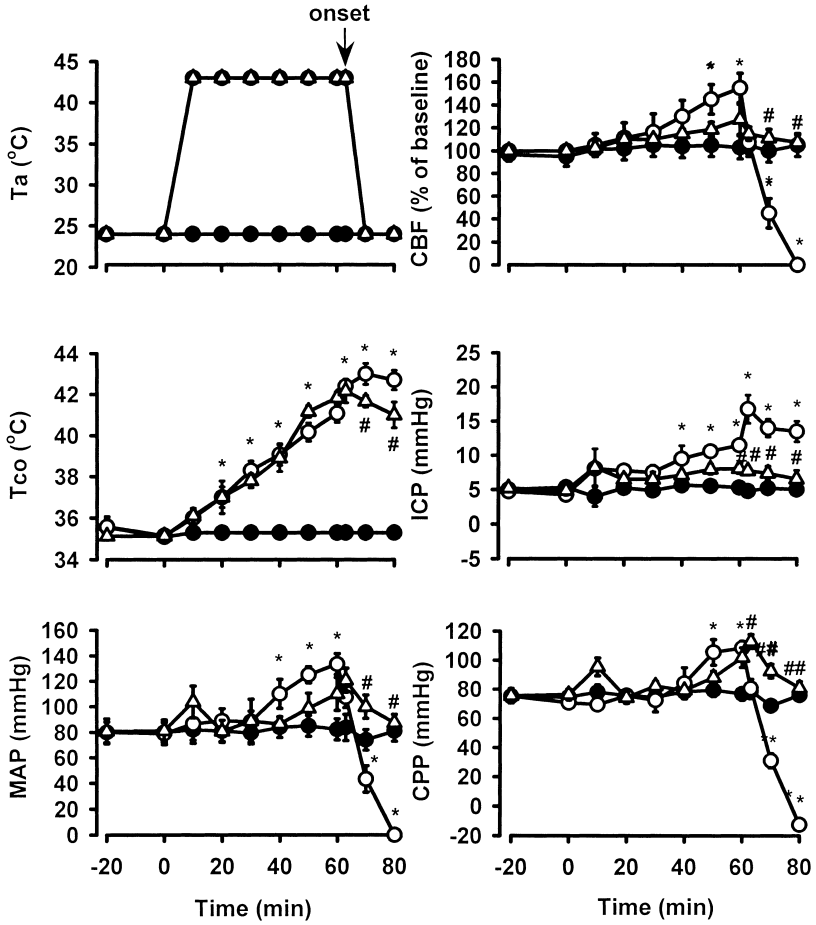

Fig. 1. Effects of heat stress (Ta 43°C for 63 min) plus 12 min after heatstroke onset on colonic temperature (Tco), mean arterial pressure (MAP), cerebral blood flow (CBF), intracranial pressure (ICP), and cerebral perfusion pressure (CPP). Open circles, values at Ta of 43°C in 8 rats treated with 0.9% NaCl solution. Another 8 rats exposed to a Ta of 24°C served as a control (closed circles). Open triangles, values at Ta of 43°C in 8 rats treated with AG (30 μmol/kg, i.v.). AG or 0.9% NaCl solution was administered 30 min before the start of heat exposure. Points represent means ± S.E.M. *P<0.05, significance of difference from normothermic control values (Ta = 24°C group); ANOVA. $^{\#}P$<0.05, significance of difference from saline-treated group (at 43°C); ANOVA. The onset of heatstroke was indicated by the arrow.

brain. In addition, AG pretreatment significantly reduced the increase in extracellular levels of cerebral ischemia and damage markers (18) associated with heatstroke. These results suggest that AG protects against heatstroke-induced intracranial hypertension, hyperthermia, and cerebral ischemic injury by inhibition of cerebral iNOS-dependent NO production. Furthermore, the present results showed that AG administration was most efficient when initiated 30 min before the start of heat stress. However, delaying onset of AG injection (i.e., 0 or 30 min after the start of heat exposure) reduced the preventive efficiency on heatstroke-induced intracranial hypertension, hyperthermia, cerebral ischemia and injury markers overproduction, and cerebral iNOS-dependent NO formation.

In response to external heat stress, both depressed ventricular depolarization and decreased cardiac stroke volume produce arterial hypotension (19). Accordingly, both arterial hypotension and intracranial hypertension eventually lead to cerebral hypoperfusion after the onset of heatstroke. Cerebral hypoperfusion to below the autoregulatory level cause cerebral ischemia, which leads to neurological damage and the onset of central nervous system syndromes associated with heatstroke. As demonstrated in the present results, the prolongation of survival in rats with AG injection was found to be related to enhancement of MAP and local CBF, as well as reduction in both intracranial hypertension and cerebral neuronal damage after the onset of heatstroke. The augmentation of CBF in animals treated with AG may be brought about by higher CPP resulting from lower ICP (due to reduction in cerebral edema and

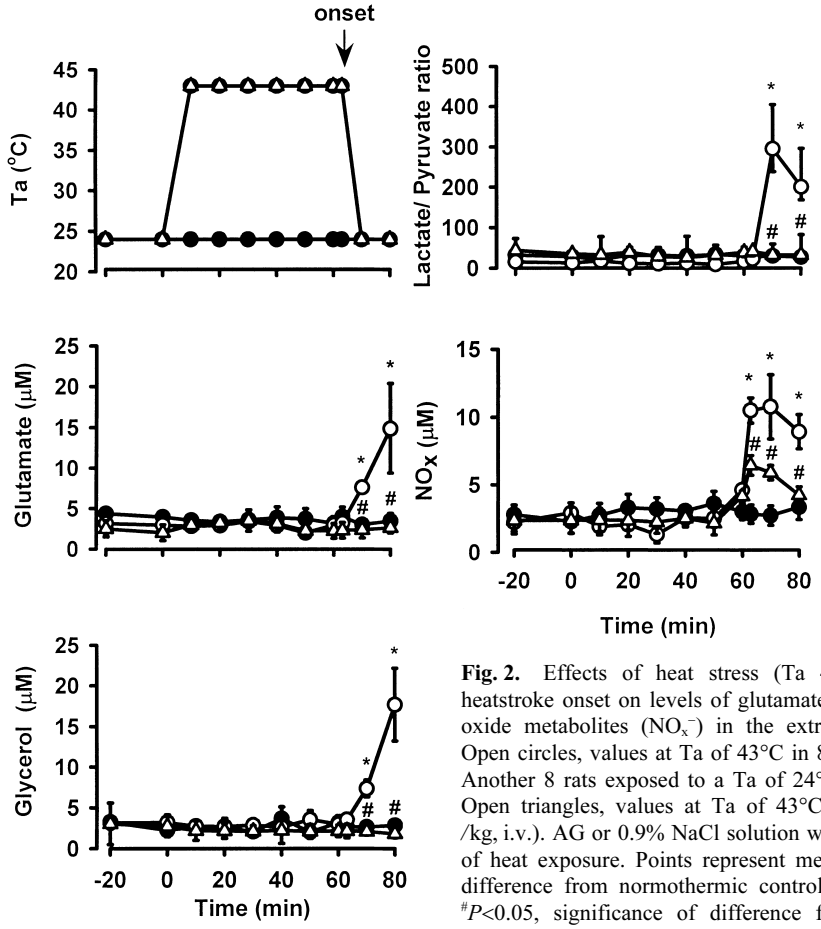

Fig. 2. Effects of heat stress (Ta 43°C for 63 min) plus 12 min after heatstroke onset on levels of glutamate, glycerol, lactate/pyruvate, and nitric oxide metabolites (NO_x^-) in the extracellular fluids of the hypothalamus. Open circles, values at Ta of 43°C in 8 rats treated with 0.9% NaCl solution. Another 8 rats exposed to a Ta of 24°C served as a control (closed circles). Open triangles, values at Ta of 43°C in 8 rats treated with AG (30 μmol /kg, i.v.). AG or 0.9% NaCl solution was administered 30 min before the start of heat exposure. Points represent means ± S.E.M. *P<0.05, significance of difference from normothermic control values (Ta = 24°C group); ANOVA. $^{\#}P$<0.05, significance of difference from saline treated group (at 43°C); ANOVA. The onset of heatstroke was indicated by the arrow.

Table 2. Effects of heat exposure (43°C for 63 min) plus 12 min after heatstroke onset on both the iNOS immunoreactivity and neuronal damage score values of the hypothalamus from normal saline-treated or AG-treated rats

Treatments	iNOS immunoreactivity (0 – 3)	Neuronal damage score (0 – 3)
Normal saline (ml/kg, i.v.)-treated rats at 24°C	0 (0, 0.75)	0 (0, 1)
Normal saline (ml/kg, i.v.)-treated rats at 43°C	2 (2, 2)*	2 (2, 2)*
AG (30 μmol/kg, i.v.)-treated rats at 43°C	1 (0.25, 1)†	1 (0, 1)†

Values represent the median with the first and third quartile in parentheses of eight rats per group. For determination of iNOS immunoreactivity and neuronal damage score, animals were killed after 63 min of heat exposure plus 12 min after heatstroke onset. The data were evaluated by a Wilcoxon signed rank test followed by the Mann-Whitney test when appropriate. *P<0.05, significance of difference from the corresponding control values (normal saline-treated rats at 24°C). $^{\dagger}P$<0.05, significance of difference from the corresponding control values (normal saline-treated rats at 43°C).

cerebrovascular congestion) and higher MAP during the development of heatstroke (19).

Both clinical and experimental evidence implicate that cooling may attenuate brain injury and improve outcome in stroke victims (20, 21). Our previous results (15) also demonstrated that cooling immediately after the onset of heatstroke reduced the heatstroke-induced circulatory shock, cerebral ischemia, neuronal damage, and surge of tissue ischemia and damage markers in brain; and this resulted in prolongation of the survival time. Delaying the onset of cooling reduced the therapeutic efficiency. Therefore, in the present

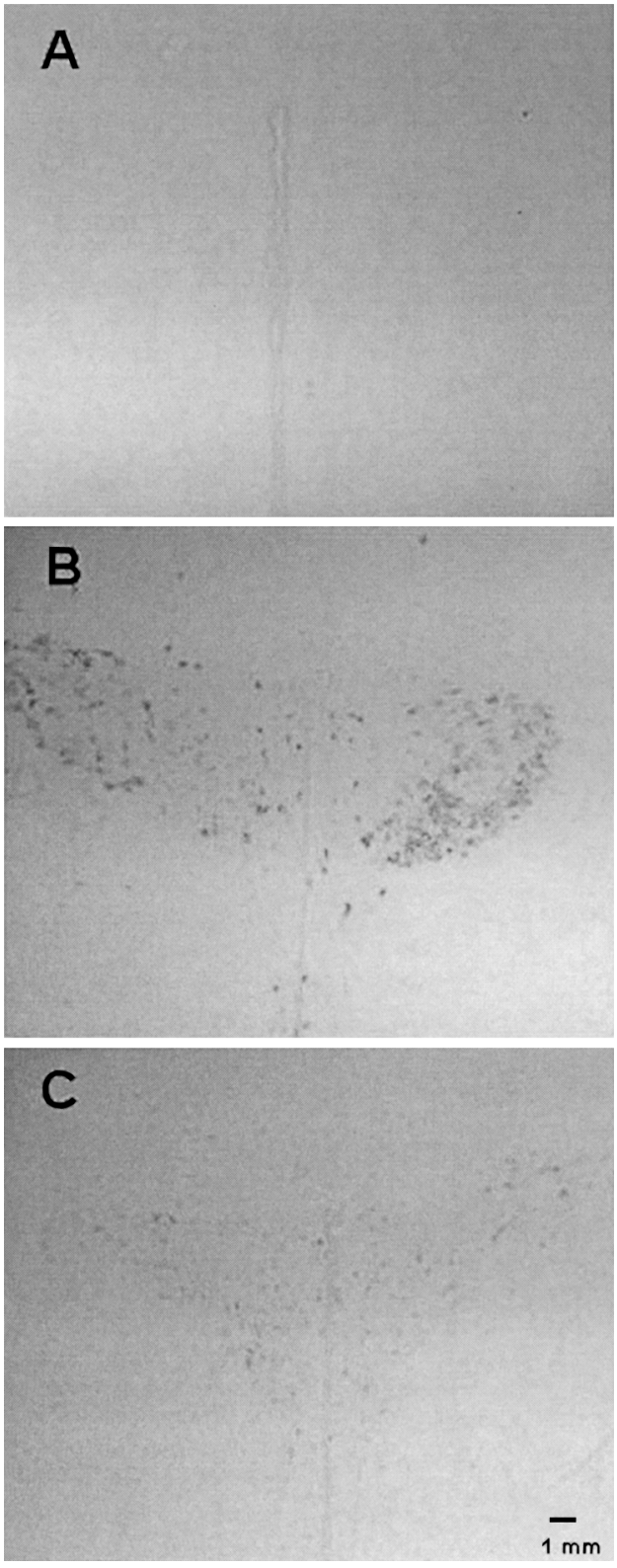

Fig. 3. Photomicrographs of iNOS staining of the hypothalamus of a normothermic control rat (A), a heatstroke rat receiving normal saline (B), and a heatstroke rat receiving AG (C). The animals were killed at 12 min after the onset of heatstroke or the equivalent time for the normothermic controls. Bar, 1 mm.

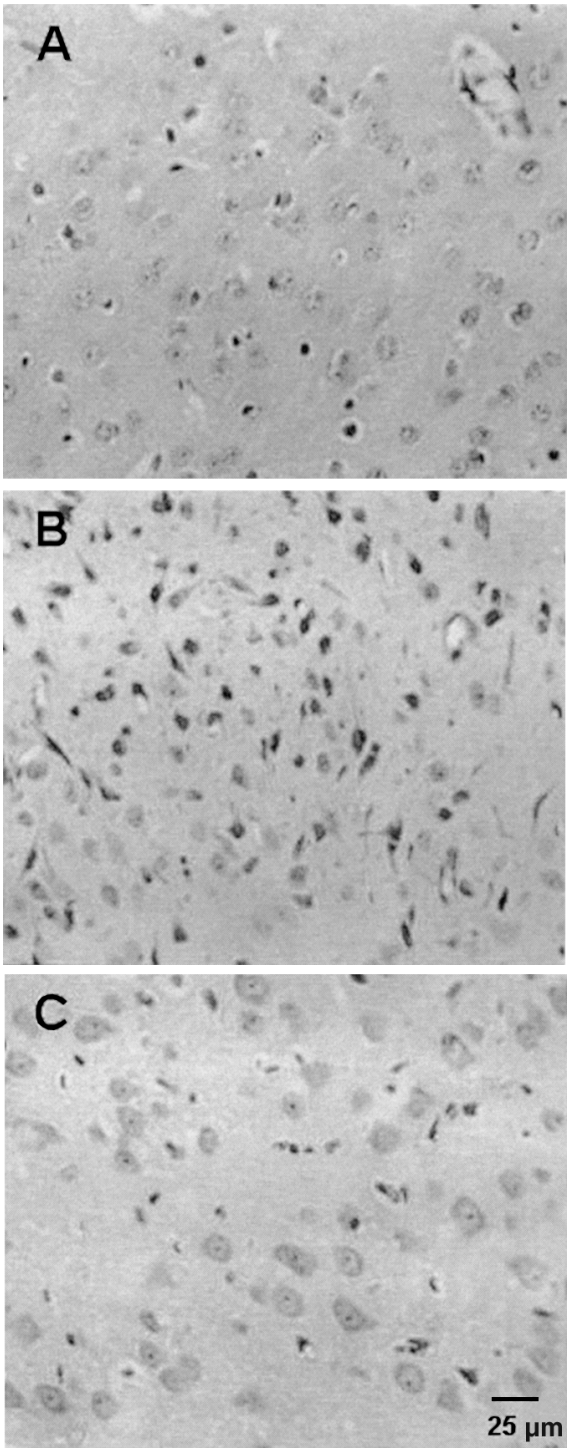

Fig. 4. Histological examination of neuronal damage. A: Photomirographs of the hypothalamus of a normothermic control rat injected with normal saline. B: Hypothalamus of a heatstroke rat injected with normal saline 30 min before the start of heat exposure. C: Hypothalamus of a heatstroke rat injected with AG (30 μmol /kg, i.v.) 30 min before the start of heat exposure. Twelve minutes after the onset of heatstroke, the hypothalamus of the rat not injected with AG showed cell shrinkage, pyknosis of the nucleus, and loss of Nissl substance (B). However, following the injection of AG, neuroprotection was induced (C). Bar, 25 μm.

results, AG may attenuate circulatory shock and cerebral ischemic injury by reducing hyperthermia during heatstroke.

It has been shown that an endotoxin given systemically can elicit an increase of iNOS-dependent NO production in the nucleus tractus solitarii (NTS) and induce arterial hypotension (22). Exposure of animals to a hot environment induces heatstroke that is characterized by arterial hypotension, endotoxemia, cerebral ischemia (8, 10), and reduced baroreceptor reflex response (23). In the present results, the heatstroke-induced ischemia, iNOS overexpression, and NO overproduction in rat hypothalamus can be suppressed by pretreatment with AG. As mentioned in our previous results (8, 10, 18, 19), ischemia occurred not only in the hypothalamus, but also in other brain structures, during heatstroke. Thus, inhibition of the iNOS-dependent NO formation in the brain with AG may alleviate arterial hypotension as well as cerebral ischemia exhibited during the onset of heatstroke by potentiating both the sensitivity and capacity of the baroreceptor reflex response.

Inducible NOS under the pathological condition can be expressed in most tissues, including neurons, astrocytes, and endothelial cells (24). In rat brain, iNOS protein is detectable 12 h after cerebral ischemia (25). AG, administered 24 h after the ischemic insults, results in reduced infarct volumes compared with vehicle-treated controls (26). In a recent report, the appearance of reactive glial cells in brain was observed after the onset of heatstroke (27) as measured by the increased number of GFAP (grial fibrillary acidic protein)-reactive cells. In the present study, AG might reduce gliosis and NO release in rat brain with heatstroke. NO generated from iNOS in endotoxic shock plays an important role in vascular hyporeactivity and tissue damage through its cytotoxic function (28, 29). Hom et al. (28) reported that endotoxin could induce expression of iNOS mRNA and protein in the brain. Further evidence suggests that glial cells may contribute to iNOS expression under inflammatory conditions (30). A progressive increase of NO production in the NTS occurred after LPS stimulation. This effect was antagonized by pretreatment with AG, which indicates an iNOS-dependent NO formation in the NTS by systemic lipopolysaccharide (22). The activation of the *N*-methyl-D-aspartate (NMDA) receptor and formation of NO by iNOS may directly signal the mitochondrial release of cytochrome c or formation of peroxynitrite ($ONOO^-$), and subsequent hydroxyl radical production can directly damage lipids, proteins, and DNA and lead to cell death, most likely necrosis (31). It seems that the similar mechanisms in the hypothalamus can be applied partially to explain the pathogenesis of cerebral ischemic insults resulting from heatstroke. For example, heatstroke triggers the increase in extracellular glutamate (18), interleukin-1 (IL-1), and tumor necrosis factor (TNF) (32) in brain. A blockade of the NMDA receptors using MK801 (an NMDA-receptor channel blocker) or the IL-1 receptors using a IL-1-receptor antagonist would be beneficial in preventing heatstroke-induced arterial hypotension and cerebral ischemia (8, 32). The formation of reactive oxygen species may be triggered by glutamate, IL-1, TNF, or NO. In fact, Hall and colleagues have concluded that hyperthermia stimulates xanthine oxidase production of reactive oxygen species that activate metals and limit heat tolerance by promoting circulatory and intestinal barrier dysfunction (2). In addition, overproduction of NO may contribute to the splanchnic vasodilation that precedes vascular collapse with heatstroke. Our previous (10) results has also demonstrated that accumulation of large amounts of reactive oxygen species in rat brain structures including the hypothalamus with heatstroke-induced cerebral ischemia. Accordingly, AG may attenuate the excessive accumulation of reactive oxygen and nitrogen species in the peripheral blood stream as well as several brain structures and result in attenuation of arterial hypotension, as well as intracranial hypertension during heatstroke onset. AG may augment CBF by attenuating arterial hypotension and intracranial hypertension as shown in the present results.

In summary, the present results indicate that AG protects against heatstroke-induced intracranial hypertension and cerebral ischemic injury by inhibition of iNOS-dependent NO formation in brain.

Acknowledgments

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