ABSTRACT—We assess the effects of ipsapirone (a 5-HT1A receptor agonist), ketanserin (a 5-HT2A receptor antagonist), (–)-pindolol (a 5-HT1A receptor antagonist), and DOI (a 5-HT2A receptor agonist) on heatstroke in a rat model. Animals, under urethane anesthesia, were exposed to high ambient temperature of 42°C until mean arterial pressure and local cerebral blood flow in the striatum began to decrease, which was arbitrarily defined as the onset of heatstroke. Normothermic controls were exposed to room temperature of 24°C. In rats treated with normal saline immediately before the initiation of heat stress, the values for survival time were found to be 21 to 25 min. Systemic administration of ipsapirone (10 mg/kg) or ketanserin (2 mg/kg) immediately before the initiation of heat stress significantly increased the survival time to new values of 92 to 104 min. Combined treatment with ipsapirone and ketanserin had additive effects (survival time of 156–194 min). In contrast, systemic administration of (–)-pindolol (2 mg/kg) or DOI (2 mg/kg) significantly decreased the survival time to new values of 2 to 3 min. In vehicle-treated heatstroke rats, the values for core temperature, intracranial pressure, and the extracellular levels of cellular ischemia (e.g., glutamate and lactate/pyruvate ratio) or damage (e.g., glycerol) markers and neuronal damage scores in striatum were significantly higher than those of normothermic controls. On the other hand, the values for mean arterial pressure, cerebral perfusion pressure, cerebral blood flow, and brain partial pressure of O2 were significantly lower than those of normothermic controls. The heatstroke-induced hyperthermia, arterial hypotension, intracranial hypertension, cerebral hypoperfusion and hypoxia, and increased levels of cellular ischemia and damage markers in striatum were all significantly attenuated by prior administration of ipsapirone or ketanserin. The present results strongly suggest that previous activation of 5-HT1A receptors or antagonism of 5-HT2A receptors protects against heatstroke by reducing circulatory shock and cerebral ischemia, whereas prior antagonism of 5-HT1A receptors or activation of 5-HT2A receptors exacerbates heatstroke.

KEYWORDS—Arterial hypotension, hyperthermia, heat stress, hypoxia, serotonin

INTRODUCTION

When rodents are exposed to a hot environment, heatstroke animals display hyperthermia, arterial hypotension, intracranial hypertension, decreased cerebral perfusion, and cerebral ischemia and injury (1–2). In addition, degeneration of cerebral neurons with replacement by neuronal loss, brain perivascular edema, swollen neuronal and glial cells, axonal swelling, and synaptic damage occurred during heatstroke (3, 4). The heatstroke-induced cerebral ischemia and damage and arterial hypotension were attenuated by serotonin depletion produced by 5,7-dihydroxy-tryptamine (1).

Other lines of evidence have accumulated to indicate that the 5-HT2A- and 5-HT1A-receptor activation in the hypothalamus mediate the hyperthermic and hypothermic action, respectively, in rats (5). An increase in core temperature and hypothalamic 5-HT efflux of rats occurred after electrical stimulation of raphe nuclei (6). Local perfusion of the hypothalamus with dialysis solution containing 5-hydroxytryptophan (a 5-HT precursor), fluoxetine (a 5-HT-reuptake inhibitor), or high potassium significantly increased core temperature and extracellular concentrations of 5-HT in the hypothalamus (7). Systemic administration of 5-HT2A-receptor agonists such as DOI, MK-212, or quipazine cause hyperthermia (8–11). The hyperthermia induced by 5-HT2A-receptor agonists was antagonized by treatment with 5-HT2A-receptor antagonists such as ketanserin, LY53857, mianserin, or ritanserin or with a 5-HT1A-receptor antagonist such as spiperone. Reciprocally, core temperature and extracellular levels of 5-HT in hypothalamus decreased with a dialysis solution containing tetradoxotin, zero calcium concentration, or systemic administration of 8-OH-DPAT (a 5-HT1A receptor agonist) (7). The hypothermia and decreased 5-HT efflux in brain produced by systemic administration of 8-OH-DPAT can be reversed by (–)-pindolol (a 5-HT1A receptor antagonist) (10, 12). However, relatively little evidence is available (4) about the effects of 5-HT1A and 5-HT2A receptor agonists and antagonists on heatstroke in the rat.

To deal with the question in the present study, we compared the survival time (interval between the onset of heatstroke and animal death) and/or the temporal profiles of mean arterial pressure (MAP), intracranial pressure (ICP), cerebral perfusion pressure (CPP), cerebral blood flow (CBF), brain partial pressure (PO2), brain temperature (Tb), core temperature (Tco), and brain levels of glutamate, glycerol, lactate/pyruvate ratio, and
neuronal damage in rats with or without prior administration of ipsapirone (a 5-HT1A receptor agonist), (−)-pindolol (a 5-HT1A receptor antagonist), DOI (a 5-HT2A receptor agonist), and ketanserin (a 5-HT2A receptor antagonist) during heatstroke.

**MATERIALS AND METHODS**

**Experimental animals**

Adult Sprague-Dawley rats (weighing 250 ± 10 g) were obtained from the Animal Resource Center of the National Science Council of the Republic of China (Taipei, Taiwan). The animals were housed four in a group at an ambient temperature of 22°C ± 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) in accordance with the guide for the Care and Use of Laboratory Animals of the National Institutes of Health as well as the guidelines of the Animal Welfare Act. Adequate anesthesia was maintained to different extents induced by isoflurane throughout all experiments (approximately 8 h) by a single intraperitoneal dose of urethane (1.4 g/kg body weight). At the end of the experiments, control rats and any rats that had survived heatstroke were killed with an overdose of urethane.

**Induction of heatstroke**

Before induction of heatstroke, the core temperature of urethane-anesthetized rat was maintained at approximately 36°C with a folded heating pad, except during heat stress, which was at a room temperature of 24°C. Heatstroke was induced by increasing the temperature of the folded heating pad to 42°C with circulating hot water. The moment at which the MAP dropped to 25 mmHg from the peak level was taken to be the onset of heatstroke (3, 13). Immediately after the onset of heatstroke, 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 heatstroke (interval between the start of heat exposure and the onset of heatstroke) was found to be 8 ± 2 min for the vehicle-treated heatstroke group (n = 8).

**Physiologic parameters and survival times (intervals between the initiation of heatstroke and animal death)** were then observed to 450 min (or the end of experiments). For comparison with the vehicle-treated heatstroke group, all drug-treated heatstroke group animals were exposed to heat for exactly 87 min and were then allowed to recover at room temperature (24°C).

**Experimental group**

The animals under urethane anesthesia were divided into the following groups. In the normothermic group (n = 8), the core temperature was maintained at approximately 36°C with a folded heating pad at a room temperature of 24°C throughout the entire experiment. In the vehicle-treated heatstroke groups, the animals were treated with a dose of normal saline intravenously (1 mL per kg body weight) immediately at the onset of heat exposure. In the drug-treated heatstroke groups, the animals received an i.v. dose of ipsapirone (10 mg/mL per kg body weight; Sigma Chemical, St. Louis, MO), ketanserin (2 mg/mL per kg body weight; Sigma Chemical), (−) pindolol (2 mg/mL per kg body weight; RBI, Natik, MA), or DOI (2 mg/mL per kg body weight; RBI) immediately at the onset of heat exposure.

**Surgery and physiological parameter monitoring**

The right femoral artery and vein of rats were cannulated with polyethylene tubing (PE 50) under urethane anesthesia for blood pressure monitoring and drug administration. The animals were positioned in a stereotaxic apparatus (Kopf 1400; Grass Instrument Co., Quincy, MA) to insert probes for measurement of ICP. ICP was monitored with a Statham P23AC transducer via a 20-gauge stainless steel needle probe (diameter, 0.90 mm; 38 mm) that was introduced into the right lateral cerebral ventricle according to the stereotaxic coordinates of Paxinos and Watson (14): 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. Tco was measured with a device (measuring PO2 and temperature at two sites simultaneously), whereas OxyFlo was a two-channel laser Doppler perfusion monitoring instrument. The OxyFlo has been designed to operate in conjunction with the OxyLite. The combination of these two instruments provides simultaneous tissue blood flow, oxygenation, and temperature data. Under urethane anesthesia, the animals were placed in a stereotaxic apparatus, and the combined probe was implanted into the striatum using the atlas and coordinates of Paxinos and Watson (14). The probe calibration parameters were transferred from the probe packaging to the OxyLite instrument using the bar code wand. For each PO2 input on the OxyLite front panel, 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: to automatically compensate the PO2 measurement and to continuously monitor tissue temperature. The OxyLite is a laser Doppler flowmeter whose primary purpose is to measure real-time microvascular red blood cell perfusion. Laser Doppler signals were recorded in 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 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 before removal from the skull. The brain was removed and embedded in paraffin blocks. Serial (10 μm) sections through the striatum were stained with hematoxylin and eosin for microscopic evaluation. The extent of cerebral neuronal damage was scored on a scale of 0 to 3, modified from previous studies (15). The score was determined by examining the extent of neuronal damage in gray matter, only areas other than those invaded by probes were assessed.

**Statistical analysis**

Data are presented as means ± SEM. Repeated-measures analysis of variance was conducted to test the treatment-by-time interactions and the effect of treatment over time on each score. The 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 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.

**Measurement of extracellular glutamate, glycerol, and lactate/pyruvate in the corpus striatum**

Each animal was anesthetized with urethane administered intraperitoneally. The animal’s head was mounted in a stereotaxic apparatus (Davis Kopf Instruments, Tujunga, CA) with the nose bar positioned 3.3 mm below the horizontal line. After making 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 striatum according to the atlas and coordinates of Paxinos and Watson (14). The coordinates for the right striatum were: A, interaural 9.7 mm; L, 2.0 mm from the midline; and H, 4.5 mm from the top of the skull. As described previously (15), 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 Na+ 147 mmol/L, K+ 4.0 mmol/L, Ca2+ 2.3 mmol/L, and CI− 156 mmol/L, and the dialysates were sampled multiprofusively. The dialysates were collected every 10 min in a CMA/140 fraction collector. Aliquots of dialysates (5 μL) were injected into a CMA 600 Microdialysis (Carnegie Medicine, Stockholm, Sweden) analyzer for measurement of lactate, glycerol, pyruvate, and glutamate. All reagents required for analysis were obtained from CMA Microdialysis.

**Measurements of CBF, brain O2, and Tb**

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 Opttronix, Oxford, UK) is a two-channel device (measuring PO2 and temperature at two sites simultaneously), whereas OxyFlo 2000 is a two-channel laser Doppler perfusion monitoring instrument. The OxyFlo has been designed to operate in conjunction with the OxyLite. The combination of these two instruments provides simultaneous tissue blood flow, oxygenation, and temperature data. Under urethane 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 (14). The probe calibration parameters were transferred from the probe packaging to the OxyLite instrument using the bar code wand. For each PO2 input on the OxyLite front panel, 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: to automatically compensate the PO2 measurement and to continuously monitor tissue temperature. The OxyLite is a laser Doppler flowmeter whose primary purpose is to measure real-time microvascular red blood cell perfusion. Laser Doppler signals were recorded in 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.
RESULTS

Ipsapirone and ketanserin improve survival during heatstroke

Table 1 summarizes the survival time values for normothermic controls, vehicle-treated heatstroke rats, and drug-treated heatstroke rats. It can be seen from the table that survival time values during heatstroke for rats treated with normal saline were found to be about 21 to 25 min. Treatment with ipsapirone (10 mg/kg) or ketanserin (2 mg/kg) significantly increased survival time to new values of 92 to 104 min. Combined treatment with ipsapirone and ketanserin had additive benefits (survival time 156–194 min). In contrast, treatment with (−)-pindolol (2 mg/kg) or DOI (2 mg/kg) significantly decreased survival time to new values of 3 to 5 min.

Ipsapirone and ketanserin attenuate heatstroke-induced physiological dysfunction and cerebral ischemia and damage

Tables 2 and 3 summarize the effects of heat exposure (42°C for 87 min) on several physiological and biochemical parameters during heatstroke. Data are mean ± SEM of eight animals per group. Vehicles or drugs were administered 87 min before initiation of heat exposure.

Table 2: Effects of heat stress (ambient temperature of 42°C for 87 min) on MAP, ICP, CPP, CBF, Brain PO2, and Tb of normothermic controls, saline-treated heatstroke rats, and drug-treated heatstroke rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>MAP (mmHg)</th>
<th>ICP (mmHg)</th>
<th>CPP (mmHg)</th>
<th>CBF (BPU)</th>
<th>Brain PO2 (mmHg)</th>
<th>Tb (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normal saline-treated normothermic controls</td>
<td>86 ± 4</td>
<td>5 ± 2</td>
<td>81 ± 3</td>
<td>277 ± 41</td>
<td>11 ± 2</td>
<td>36.0 ± 0.2</td>
</tr>
<tr>
<td>2. Normal saline-treated heatstroke rats</td>
<td>87 ± 3</td>
<td>6 ± 2</td>
<td>81 ± 3</td>
<td>263 ± 39</td>
<td>12 ± 2</td>
<td>36.1 ± 0.3</td>
</tr>
<tr>
<td>3. Ipsapirone-treated heatstroke rats</td>
<td>85 ± 4</td>
<td>6 ± 2</td>
<td>79 ± 4</td>
<td>269 ± 38</td>
<td>13 ± 2</td>
<td>36.2 ± 0.2</td>
</tr>
<tr>
<td>4. Ketanserin-treated heatstroke rats</td>
<td>41 ± 3*</td>
<td>33 ± 4*</td>
<td>9 ± 2*</td>
<td>109 ± 23*</td>
<td>4 ± 2*</td>
<td>41.7 ± 0.4*</td>
</tr>
<tr>
<td>5. Ketanserin-treated heatstroke rats</td>
<td>84 ± 3</td>
<td>6 ± 2</td>
<td>78 ± 4</td>
<td>303 ± 55</td>
<td>12 ± 2</td>
<td>36.2 ± 0.2</td>
</tr>
<tr>
<td>6. (−)-Pindolol-treated heatstroke rats</td>
<td>67 ± 4</td>
<td>1 ± 3</td>
<td>56 ± 3</td>
<td>226 ± 26</td>
<td>10 ± 2</td>
<td>39.3 ± 0.3*</td>
</tr>
<tr>
<td>7. DOI (2 mg/kg, i.v.)-treated heatstroke rats</td>
<td>83 ± 4</td>
<td>5 ± 2</td>
<td>78 ± 3</td>
<td>285 ± 34</td>
<td>13 ± 2</td>
<td>36.3 ± 0.2</td>
</tr>
</tbody>
</table>

*P < 0.05 in comparison with group 1; †P < 0.05 in comparison with group 2; 2: P < 0.05 in comparison with group 3 or 4 (analysis of variance followed by Duncan’s test). All saline-treated or drug-treated heatstroke rats had heat exposure withdrawn exactly at 87 min and were then allowed to recover at room temperature (24°C). Data are mean ± SEM of eight animals per group. Vehicles of drugs were administered 87 min before initiation of heat exposure.

Table 3: Effects of heat stress (ambient temperature of 42°C for 87 min) on mean Tco, MAP, and extracellular concentrations of glutamate, glycerol, and lactate/pyruvate in the striatum of normothermic controls, saline-treated heatstroke rats, or drug-treated heatstroke rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Tco (°C)</th>
<th>MAP (mmHg)</th>
<th>Glutamate (μmol/L)</th>
<th>Glycerol (μmol/L)</th>
<th>Lactate/Pyruvate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline-treated rats at 24°C:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min after testing</td>
<td>36.1 ± 0.2</td>
<td>89 ± 3</td>
<td>0.98 ± 0.52</td>
<td>6 ± 2</td>
<td></td>
</tr>
<tr>
<td>100 min after testing</td>
<td>36.0 ± 0.3</td>
<td>85 ± 4</td>
<td>0.97 ± 0.63</td>
<td>6 ± 2</td>
<td></td>
</tr>
<tr>
<td>2. Saline-treated rats at 42°C:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min after heat exposure</td>
<td>36.1 ± 0.2</td>
<td>84 ± 4</td>
<td>0.93 ± 0.55</td>
<td>6 ± 2</td>
<td></td>
</tr>
<tr>
<td>100 min after heat exposure</td>
<td>42.7 ± 0.3*</td>
<td>42 ± 4*</td>
<td>94 ± 18*</td>
<td>29 ± 3*</td>
<td>254 ± 44*</td>
</tr>
<tr>
<td>3. Ipsapirone-treated rats at 42°C:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min after heat exposure</td>
<td>36.2 ± 0.2</td>
<td>87 ± 3</td>
<td>0.92 ± 0.44</td>
<td>6 ± 2</td>
<td></td>
</tr>
<tr>
<td>100 min after heat exposure</td>
<td>40.5 ± 0.2†</td>
<td>70 ± 4†</td>
<td>37 ± 8†</td>
<td>68 ± 15†</td>
<td></td>
</tr>
<tr>
<td>4. Ketanserin-treated rats at 42°C:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 min after heat exposure</td>
<td>36.4 ± 0.3</td>
<td>82 ± 4</td>
<td>0.89 ± 0.51</td>
<td>6 ± 2</td>
<td></td>
</tr>
<tr>
<td>100 min after heat exposure</td>
<td>40.6 ± 0.3†</td>
<td>75 ± 3†</td>
<td>32 ± 6†</td>
<td>64 ± 16†</td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05 in comparison with group 1; †P < 0.05 in comparison with group 2 (analysis of variance followed by Duncan’s test). All saline-treated or drug-treated heatstroke rats had heat exposure withdrawn exactly at 87 min and were then allowed to recover at room temperature (24°C). Data are mean ± SEM of eight animals per group. Vehicles or drugs were administered 87 min before initiation of heat exposure.
in rats treated with normal saline, rats treated with drugs, and normothermic controls. In vehicle-treated heatstroke groups, the ICP, Tb, and cellular levels of glutamate, glycerol, and lactate/pyruvate were significantly higher at 100 min after the start of heat exposure than they were for normothermic controls. In contrast, the values for MAP, cerebral perfusion pressure, CBF, and brain PO2 were all significantly lower than those of normothermic controls. Treatment with ipsapirone or ketanserin immediately after initiation of heat exposure significantly attenuated the heat stress-induced arterial hypotension, intracranial hypertension, cerebral hypoperfusion, and cerebral hypoxia and increased levels of cellular ischemia and damage markers in striatum.

**Ipsapirone and ketanserin attenuate heatstroke-induced neuronal damage**

Table 4 summarizes the neuronal damage scores of striatum in normothermic rats, vehicle-treated heatstroke rats, ipsapirone-treated heatstroke rats, and ketanserin-treated heatstroke rats. It was found that the scores for neuronal damage in heatstroke rats treated with vehicle solutions immediately after initiation of heat exposure (median [Q1, Q3], [2, 2]) were all significantly greater \( P < 0.05 \) than those for the normothermic controls (median [Q1, Q3], 0 [0, 0.75]). However, the neuronal damage scores for heatstroke rats treated with ipsapirone or ketanserin (median [Q1, Q3], 0 [0, 1]) were all significantly lower \( P < 0.05 \) than those for the heatstroke controls. Figure 1 shows that heatstroke-induced cell body shrinkage, pyknosis of the nucleus, and loss of Nissl substance in the striatum were attenuated with ipsapirone.

**DISCUSSION**

The present findings demonstrate that the administration of ipsapirone (a 5-HT1A receptor agonist) or ketanserin (a 5-HT2A receptor antagonist) improves survival during heatstroke. On the other hand, the administration of \((-\)-pindolol (a 5-HT1A receptor antagonist) or DOI (a 5-HT2A receptor agonist) worsens survival during heatstroke. Furthermore, combined pretreatment with ipsapirone and ketanserin produced additive benefits during heatstroke. As described in the former section, the 5-HT2A and 5-HT1A receptor activation in the hypothalamus mediates the hyperthermic and hypothermic action, respectively, in the rat. Therefore, it is inferred that ipsapirone or ketanserin may improve survival during heatstroke by reducing hyperthermia.

Our present and previous (15, 17, 18) results demonstrate that in addition to inducing hyperthermia, the heatstroke rats

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**TABLE 4. Effects of heat stress (ambient temperature of 42°C for 87 min) on neuronal damage in the striatum of normothermic controls, saline-treated heatstroke rats, or drug-treated heatstroke rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Neuronal damage score</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Saline-treated rats at 24°C</td>
<td>0 (0, 0.75)</td>
</tr>
<tr>
<td>2. Saline-treated rats at 42°C</td>
<td>2 (2, 2)*</td>
</tr>
<tr>
<td>3. Ipsapirone (10 mg/kg, i.v.)-treated rats at 42°C</td>
<td>0 (0, 1)*</td>
</tr>
<tr>
<td>4. Ketanserin (2 mg/kg, i.v.)-treated rats at 42°C</td>
<td>0 (0, 1)*</td>
</tr>
</tbody>
</table>

Data for eight rats per group presented as "median" with "Q1 And Q3" in parenthesis. For the determination of neuronal damage score, animals were killed 100 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 (analysis of variance followed by Duncan’s test). All saline-treated or drug-treated heatstroke rats had heat exposure exactly at 87 min and were then allowed to recover at room temperature (24°C). Vehicles or drugs were administered 87 min before initiation of heat exposure.
displayed intracranial hypertension, arterial hypotension, cerebral hyperperfusion, cerebral ischemia and hypoxia, and increased levels of cellular ischemia (e.g., glutamate and lactate/pyruvate ratio) and damage (e.g., glycerol) markers in striatum. In the present results, we further reported that pretreatment with systemic administration of ipsapirone or ketanserin greatly attenuates the above-mentioned reactions during heatstroke. In fact, after the onset of heatstroke, ischemic injury is noted to occur in different brain structures, including striatum, hypothalamus, cortex, and thalamus (1, 19). In the present study, the striatum is chosen as a representative region for measurement of blood flow, PO2, and neuronal damage during heatstroke. The present results strongly suggest that prior antagonism of 5-HT2A receptors or prior activation of 5-HT1A receptors protects against circulatory shock and cerebral ischemia during heatstroke. The contention is supported by several previous findings. For example, ipsapirone applied 1 h after middle cerebral vessel occlusion leads to a reduction in cortical infarct volumes by about 50% compared with corresponding controls (P < 0.05) (20). Pretreatment with ketanserin markedly ameliorates brain edema in rats attendant with heatstroke (4).

The pressor and resistance responses to constant infusions of several vasopressor agents after arterial pressure began falling in hyperthermic animals are significantly lower than with infusions into normothermic animals (21). Our previous results further show that constant infusions of a vasopressor agent phenylephrine after the onset of heatstroke failed to maintain appropriate levels of arterial pressure and result in no protection against heatstroke (18). These indicate that hyperthermia in association with onset of heatstroke disrupts adrenergic function and does not keep the maintenance of arterial pressure. In the present results, ipsapirone or ketanserin may have attenuated arterial hypotension by reducing hyperthermia during heatstroke. In fact, activation of the 5-HT2A and 5-HT1A receptors in the hypothalamus is believed to mediate the hyperthermic and hypothermic action, respectively, in rats (5, 22). Thereafter, in the present results, activation of 5-HT1A receptors with ipsapirone or antagonism of 5-HT2A receptors with ketanserin in the hypothalamus may have reduced the hyperthermia that occurred during heatstroke onset; a lesser extent of body hyperthermia would help to maintain normal adrenergic receptor sensitivity and appropriate levels of MAP during heatstroke.

In addition, in the current results, the maintenance of appropriate levels of CBF and oxygenation in animals treated with ipsapirone or ketanserin may be brought about by higher cerebral perfusion pressure resulting from lower intracranial pressure (from reduction in cerebral edema and cerebrovascular congestion) and higher arterial pressure during the development of heatstroke (23). Indeed, the heatstroke-induced arterial hypotension, intracranial hypertension, cerebral ischemia and damage, and increased cerebral levels of 5-HT were significantly ameliorated by depleting brain 5-HT levels with 5, 7-dihydroxytryptamine (1). Sharma and colleagues (4) reported that pretreatment with ketanserin markedly reduced brain edema during heatstroke in rats. Thus, it appears that the presence of brain 5-HT accumulation is important for the development of cerebral ischemia and neuronal death during heatstroke. One potential mechanism of action is that 5-HT overproduction reduces MAP and increases intracranial pressure. These actions attenuate CBF and result in injury to neurons. As depicted in our previous results (5, 22), 5-HT1A receptor agonists including ipsapirone, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT), buspirone, gepirone, and Bay R1531 decrease cortical infarct size in rat focal ischemia produced by occlusion of the middle cerebral artery (20). Microdialysis data revealed that 8-OH-DPAT and other 5-HT1A agonists acted through presynaptic 5-HT1A receptors to decrease brain 5-HT release (7, 23). On the other hand, ketanserin and other 5-HT2A antagonists may act through postsynaptic 5-HT2A receptors to block the 5-HT reuptake (5) and to attenuate cerebral ischemia.

REFERENCES