Involvement of interleukin-1 receptor mechanisms in development of arterial hypotension in rat heatstroke

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Lin, M. T., H. H. Liu, and Y. L. Yang. Involvement of interleukin-1 receptor mechanisms in development of arterial hypotension in rat heatstroke. Am. J. Physiol. 273 (Heart Circ. Physiol. 42) H2072–H2077, 1997.—Rats, under urethan anesthesia, were exposed to a high ambient temperature (42°C) to induce heatstroke and to assess the hemodynamic changes associated with heatstroke. Compared with normothermic controls, rats with heatstroke showed higher values of colonic temperature, heart rate, and plasma levels of interleukin (IL-1) but lower values of R wave amplitude, P-R and Q-T intervals, systolic wave amplitude, diastolic and dicrotic wave duration, mean arterial pressure, stroke volume, and cardiac output. Animals injected intravenously with an IL-1-receptor antagonist at the time of heatstroke induction were protected from some of the cardiovascular effects of heatstroke, such as depressed ventricular depolarization, decreased stroke volume, decreased cardiac output, and arterial hypotension. The hemodynamic changes associated with heatstroke could be mimicked by IL-1β administration. Other cardiovascular parameters such as total peripheral vascular resistance were unaffected by heatstroke induction or IL-1β treatment. The results indicate that a selective decline in stroke volume or ventricular depolarization resulting from increased plasma levels of IL-1 may be an important mechanism signaling arterial hypotension or circulatory failure in rat heatstroke.

stroke volume; arterial hypotension; interleukin-1; cardiac output

HEATSTROKE is a complex clinical picture characterized by severe central nervous system disturbances (such as coma and delirium), hyperpyrexia, and hot, dry skin (18). The high mortality rate of heatstroke is believed to be related to its serious complications, including adult respiratory distress syndrome, disseminated intravascular coagulation, aspiration pneumonia, pulmonary edema, circulatory and renal failure, and severe electrolyte disturbances (1, 2, 10, 13, 20, 24, 25, 28).

There is little agreement on the hemodynamic status of patients or animals with heatstroke. For example, Clowes and O'Donnell (9) reported that seven of eight patients suffering from acute heatstroke had increased cardiac index and decreased peripheral vascular resistance. Sprung (30) reported that five of seven patients with heatstroke had decreased cardiac output and increased peripheral resistance. Recently, Dahmash et al. (10) reported that among 10 heatstroke patients, cardiac output was decreased in 1, normal in 5, and increased in 4. Systemic vascular resistance, on the other hand, was low in eight patients and normal in two patients. In experimental models, animals with heatstroke had decreased mean arterial pressure (19, 23, 24) and decreased peripheral vascular resistance (21).

Evidence has accumulated to indicate that the morbidity and mortality observed in heatstroke may be related to endotoxemia and release of interleukin (IL)-1 (3, 8, 14). Immunization against bacterial endotoxin (4) or administration of antibiotics before heat stress sharply reduced mortality in experimental animals (5, 6). The arterial hypotension associated with heatstroke could be attenuated by pretreatment of animals with an antagonist of IL-1 receptors (16, 22, 23). Therefore, a potential involvement of IL-1 receptor mechanisms in the development of circulatory failure in heatstroke is suggested.

The objective of this study was to clarify the relationship between IL-1 and hemodynamic changes associated with heatstroke. Rats, under general anesthesia, were exposed to a high ambient temperature to induce heatstroke (16, 22, 23) and to assess the hemodynamic changes associated with heatstroke in control rats and in rats pretreated with an IL-1-receptor antagonist. In addition, the effects of systemic administration of IL-1β on hemodynamic function parameters were assessed in normothermic controls. Furthermore, changes in the plasma levels of IL-1 were assessed in normothermic controls and in rats with heatstroke.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats (Animal Resource Center, National Cheng-Kung University Medical College, Tainan, Taiwan), weighing 250–300 g at the start of the experiment, were housed individually in wire hanging cages in a temperature (24°C)-controlled animal colony, on a normal light-dark cycle (14:10 h; lights on at 6:00 AM). The animals had free access to food and water and were allowed to acclimatize to the light cycle and the room temperature for at least 2 wk before the experiment began.

Surgery and measurement of cardiovascular parameters. The right femoral artery and vein of rats under urethan (1.4 g/kg ip) anesthesia were cannulated with polyethylene tubing (PE-50) for monitoring of cardiovascular parameters, administration of drugs, and blood sampling. In addition,
general procedures included trachea intubation for artificial ventilation, with the rate and tidal volume adjusted to maintain an end-expiration CO₂ concentration between 3.5 and 4.0% (monitored by a Gould Capnograph Mark IV), and keeping the colonic temperature (Tₐₚ) at 38°C before heat exposure (32). Tₐₚ was measured using a copper-constantan thermocouple enclosed in polyethylene tubing, sealed at one end, and inserted 6 cm into the colon. The animals were then put in a supine position.

After the thorax was opened by a median sternotomy, the pericardium was incised and a 3.0-mm electromagnetic flowmeter probe (Gould SP 2204B) was attached to the ascending aorta to monitor the aortic blood flow. Once the animal had stabilized after this procedure, the control measurements, including phasic aortic blood flow (ml/min), mean aortic blood flow (ml/min), mean pulsatile arterial blood pressure (MAP, mmHg), mean systemic arterial blood pressure (mMAP, mmHg), and heart rate (HR, beats/min), were initiated on the anesthetized rats. All physiological data were displayed on a polygraph (Gould ES1000).

The electromagnetic flowmeter was calibrated in situ after the experiments by perfusing the ascending aorta through a peristaltic pump with heparinized blood collected from the same Tₐₚ strain of rats. The cardiac index (CI, ml·min⁻¹·100 g body wt⁻¹) was obtained by dividing cardiac output by body weight. The total peripheral resistance index (TPRI, mmHg·min⁻¹·100 g body wt⁻¹) was expressed as mMAP divided by CI. Stroke index (SI, ml·beat⁻¹·100 g body wt⁻¹) was obtained by dividing CI by HR.

Both electrocardiogram (ECG) and blood pressure (BP) waveform parameters were measured by conventional techniques as detailed elsewhere (33). In brief, four stainless steel needles were used as the recording electrodes for ECG and were connected to the ECG preamplifier (Gould model 13-4615-65) to record lead II ECG. Four 3-s periods of ECG and BP data were acquired by a locally developed microcomputer and data acquisition and analysis system during the whole course of experimentation (33). The sampling rate of the system was 1 kHz. From each selected period of data, we obtained the following waveform parameters: amplitude of P, Q, R, S, and T waves; P-R and Q-T intervals; duration of P, Q, R, and T waves; HR; amplitude of systolic and diastolic waves, duration of systolic, diastolic, and dicrotic waves; and duration of the whole BP cycle.

IL-1 bioassay. IL-1 concentration in the plasma was measured with the IL-1-dependent murine T cell line D10N4M (a kind gift from Dr. C. C. Chao, Neuromedimunity and Host Defense Laboratory, Minneapolis Medical Research Foundation, Minneapolis, MN), as previously described (7, 26). The D10N4M cells were maintained in RPMI-1640 (GIBCO BRL) with 10% fetal bovine serum (GIBCO BRL), recombinant human IL-2 (20 ng/ml, R & D), recombinant human IL-1β (40 pg/ml, R & D), 5 × 10⁻³ M 2-mercaptoethanol (Serva, Heidelberg, Germany), and concanavalin A (3.0 µg/ml; Sigma), and were fed every 3 days before being assayed. Serial rabbit serum samples or recombinant human IL-1β (50 µl, as an internal reference) were added to each well of microplates (NUNC), followed by the addition of 50 µl of washed D10N4M cells (2 × 10⁵ cells/ml). After 72 h of incubation, the cells were pulsed with 0.5 µCi of [³H]thymidine (6.7 Ci/mmol, DuPont NEN), per well for 4 h. The cells were harvested on glass fiber filters with an automatic cell harvester (Cambridge, Watertown, MA). The radioactivity incorporated was assayed in a liquid scintillation counter (LS 5000 TA, Beckman, Fullerton, CA).

Induction of heatstroke. Four groups of animals were used. In the first group, rats were exposed for 70 min to heat and received a saline injection (1 ml/kg of 0.9% saline per 1 kg body wt iv) at the start of heat exposure. Heatstroke was induced by exposing the rats, under urethan anesthesia, to an ambient temperature (Tₐₚ) of 42°C (with a relative humidity of 60%); the moment at which MAP began to decrease from its peak level was taken as the onset of heatstroke. Our previous results show that, at the moment when MAP begins to decrease from its peak level, unanesthetized animals display heatstroke symptoms including loss of sensation, decreased cerebral perfusion pressure, and unconsciousness (29), whereas animals under general anesthesia display decreased cerebral perfusion pressure, cerebral ischemia, and neuronal injury (16, 22, 23). For humane reasons, in the present study, heatstroke was induced under general anesthesia. In the second group, rats under urethan anesthesia were exposed for 70 min to heat and received IL-1 receptor antagonist (IL-1ra, 200 µg/kg iv; Synergen) at the start of heat exposure. IL-1ra was expressed in Escherichia coli using a cDNA originally isolated from adherent monocytes (12). This protein is the nonglycosylated NH₂-terminal methionyl form of the naturally occurring protein and has a molecular mass of ~17 KDa. IL-1ra blocks binding of IL-1 as well as the naturally occurring glycosylated form does. In the third group, rats under urethan anesthesia were exposed to a Tₐₚ of 24°C for at least 90 min and were given an intravenous dose of 1 ml/kg of 0.9% saline at the start of testing. In the fourth group, rats exposed to a Tₐₚ of 24°C received an intravenous dose of recombinant murine IL-1β (30 µg/kg).

Data analysis. Numerical values cited are means ± SE. Repeated-measures analysis of variance was used for factorial experiments, whereas Duncan’s multiple-range test (multiple time-point experiments) was used for post hoc multiple comparisons among means. Student’s t-test was used when only two groups were compared. The criterion for statistical significance was set at P < 0.05.

RESULTS

Tables 1, 2, and 3 summarize values for the various cardiovascular parameters collected from three groups of animals 70 min after the start of heat exposure or testing. The time-course data before, during, and after heat exposure are depicted in Figs. 1, 2, and 3. The hyperthermic rats that received saline injection 70 min after the start of heat exposure displayed higher values of Tₐₚ (Table 1) and HR (Table 2) and lower values of MAP, CI, SI (Table 1), survival time (ST; interval between onset of heatstroke and death), R wave amplitude, and P-R and Q-T intervals QT (Table 2), and amplitude of systolic and diastolic waves, duration of systolic, diastolic, and dicrotic waves, and duration of whole BP cycle (Table 3), compared with normothermic controls that received saline treatment. However, there is an insignificant difference in amplitude of P, Q, S, and T waves and duration of P, Q, R, and T waves and QRS duration (data not shown) and TPRI (Table 1) between normothermic controls and hyperthermic animals that received saline treatment. These tables and figures also show that pretreatment of rats with an intravenous dose of IL-1ra (200 µg/kg), just at the start of heat exposure, significantly attenuated the reduction in MAP, CI, SI (Table 1), R wave amplitude and P-R,
in MAP, CI, and SI in rats exposed to a Ta of 24°C. Historically, there have been two views about the pathogenesis of heatstroke, 1) direct thermal injury to the thermoregulatory centers in the brain causing thermoregulatory failure and shock (18) and 2) circulatory failure (1). From measurements of central venous Q-T, and S-T intervals (Table 2), and amplitude of systolic and diastolic waves and duration of systolic, diastolic, and dicrotic waves and the whole BP cycle (Table 3) that occurred during onset of heatstroke. Table 4 summarizes plasma IL-1 values in rats exposed for 70 min to heat (Ta 42°C) and in rats exposed to a Ta of 24°C. As can be seen from the table, the heat-exposed rats had a higher IL-1 concentration in the plasma compared with that of the controls.

In addition, as shown in Fig. 4, an intravenous dose of IL-1β (30 µg·ml−1·kg−1) produced a progressive fall in MAP, CI, and SI in rats exposed to a Ta of 24°C. Again, TPRI was not affected by the intravenous administration of IL-1β.

**DISCUSSION**

This study provides the first experimental evidence supporting the hypothesis that a selective decline of stroke volume or ventricular depolarization may be an important mechanism signaling arterial hypotension in heatstroke. As demonstrated in the present results, some cardiovascular effects, such as tachycardia, depressed ventricular depolarization, decreased stroke volume, decreased cardiac output; arterial hypotension, and facilitated pacemaker signal conduction, were associated with heatstroke induction in rats. The reduction in ventricular depolarization during heatstroke induction might be reflected by a decrease in R wave amplitude, systolic wave amplitude, diastolic wave duration, and dicrotic wave duration. The facilitated pacemaker signal conduction might be demonstrated by a decrease in P-R and Q-T intervals in rat heatstroke. However, neither TPRI nor the hemodynamic parameters related to atrial depolarization or ventricular repolarization were associated with heatstroke induction. Historically, there have been two views about the pathogenesis of heatstroke, 1) direct thermal injury to the thermoregulatory centers in the brain causing thermoregulatory failure and shock (18) and 2) circulatory failure (1). From measurements of central venous

<table>
<thead>
<tr>
<th>Groups</th>
<th>TCO, °C</th>
<th>MAP, mmHg</th>
<th>CI, ml·min⁻¹·100 g⁻¹</th>
<th>SI, µl·beat⁻¹·100 g⁻¹</th>
<th>TPRI, mmHg·min⁻¹·100 g·ml⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>37.8 ± 0.2</td>
<td>77 ± 4</td>
<td>9.5 ± 0.4</td>
<td>23.7 ± 1.3</td>
<td>7.8 ± 0.6</td>
</tr>
<tr>
<td>Rats with heat exposure + saline (0.9% iv)</td>
<td>40.4 ± 0.3*</td>
<td>30 ± 4*</td>
<td>3.8 ± 0.4*</td>
<td>9.7 ± 0.4*</td>
<td>9.1 ± 1.6</td>
</tr>
<tr>
<td>Rats with heat exposure + IL-1ra injection (200 µg/kg iv)</td>
<td>40.6 ± 0.2*</td>
<td>73 ± 3†</td>
<td>9.9 ± 0.2†</td>
<td>19.5 ± 2.0†</td>
<td>9.1 ± 1.5</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 rats for each group. TCO, colonic temperature; IL-1ra, interleukin-1-receptor antagonist; MAP, mean arterial pressure; HR, heart rate; CI, cardiac index; SI, stroke index; TPRI, total peripheral resistance index. *P < 0.05, significance of difference from corresponding control values (normothermic control group), ANOVA; †P < 0.05, significance of difference from corresponding control values (rats with heat exposure + saline treatment), ANOVA.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Aq, mV</th>
<th>PR, ms</th>
<th>QT, ms</th>
<th>HR, beats/min</th>
<th>ST, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>0.40 ± 0.02</td>
<td>52 ± 4</td>
<td>80 ± 7</td>
<td>434 ± 17</td>
<td>&gt;240</td>
</tr>
<tr>
<td>Rats with heat exposure + saline (0.9% iv)</td>
<td>0.20 ± 0.02*</td>
<td>37 ± 3*</td>
<td>66 ± 4*</td>
<td>568 ± 20*</td>
<td>20 ± 4*</td>
</tr>
<tr>
<td>Rats with heat exposure + IL-1ra (200 µg/kg iv)</td>
<td>0.40 ± 0.02†</td>
<td>38 ± 4*</td>
<td>83 ± 5†</td>
<td>547 ± 20†</td>
<td>200 ± 12†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 rats for each group. ECG, electrocardiogram; Aq, amplitude of R wave; PR, P-R interval; QT, Q-T interval; ST, survival time. *P < 0.05, significance of difference from corresponding control values (normothermic control group), ANOVA; †P < 0.05, significance of difference from corresponding control values (rats with heat exposure + saline treatment), ANOVA.

<table>
<thead>
<tr>
<th>Groups</th>
<th>DA, ms</th>
<th>Apq, mmHg</th>
<th>Apaq, mmHg</th>
<th>Dsys, ms</th>
<th>Dsys, mmHg</th>
<th>Dsys, ms</th>
<th>Dsys, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>46 ± 6</td>
<td>68 ± 6</td>
<td>22 ± 2</td>
<td>74 ± 5</td>
<td>178 ± 14</td>
<td>101 ± 10</td>
<td></td>
</tr>
<tr>
<td>Rats with heat exposure + saline (0.9% iv)</td>
<td>25 ± 2*</td>
<td>29 ± 2*</td>
<td>10 ± 1*</td>
<td>42 ± 7*</td>
<td>115 ± 12*</td>
<td>52 ± 7*</td>
<td></td>
</tr>
<tr>
<td>Rats with heat exposure + IL-1ra (200 µg/kg iv)</td>
<td>46 ± 5†</td>
<td>81 ± 6†</td>
<td>20 ± 2†</td>
<td>72 ± 6†</td>
<td>162 ± 12†</td>
<td>102 ± 12†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 rats for each group. Apq, amplitude of systolic wave; Apaq, amplitude of diastolic wave; Dsys, duration of systolic wave; Dsys, duration of a whole blood pressure cycle; DA, duration of diastolic wave; Dsys, duration of dicrotic wave. *P < 0.05, significance of difference from corresponding control values (normothermic control group), ANOVA; †P < 0.05, significance of difference from corresponding control values (rats with heat exposure + saline treatment), ANOVA.
pressure and splanchnic blood flow, Kielblock et al. (19) and Kregel et al. (21) concluded that a selective loss of compensatory splanchnic vasoconstriction may be an important mechanism signaling circulatory failure in heatstroke. However, the fall in splanchnic mesenteric artery resistance in itself may not be directly responsible for the fall in mean arterial pressure. The time course of change in splanchnic mesenteric artery resistance in relation to arterial hypotension indicates that the fall in mean arterial pressure is not caused by a decrease in total peripheral resistance (21); rather, pooling blood in the peripheral vasculature may reduce venous return and stroke volume and ultimately contribute to circulatory failure. Moreover, in the present results, we newly demonstrated that arterial hypoten-

![Fig. 1. Effects of a high ambient temperature (T_a = 42°C) on colonic temperature (T_c), mean arterial pressure (MAP), cardiac index (CI), stroke index (SI), and total peripheral resistance index (TPRI) in 8 rats treated with saline or interleukin-1 receptor antagonist (IL-1ra, 200 µg/kg iv). Points represent means ± SE. * P < 0.05, significance of difference from control values (saline group), Student’s t-test.](http://ajpheart.physiology.org/)

![Fig. 2. Effects of high T_a (42°C) on amplitude of R wave (A_r), P-R interval (PR), Q-T interval (QT), and duration of dicrotic wave (D_dic) in 8 rats receiving saline or IL-1ra (200 µg/kg iv). Points represent means ± SE. * P < 0.05, significance of difference from control values (saline group), Student’s t-test.](http://ajpheart.physiology.org/)

sion was associated with a decline in ventricular depolarization or stroke volume in heatstroke. Thus it appears that heatstroke results in circulatory shock, and the mechanism initiating the circulatory impairment is more a myocardial mechanism (31) than a peripheral mechanism (such as pooling blood in the peripheral vasculature) (11).

The present results show that heatstroke was accompanied by increased plasma levels of IL-1. Animals injected intravenously with IL-1ra at the time of heatstroke induction were protected from some of the cardiovascular effects of heatstroke such as depressed ventricular depolarization, decreased stroke volume, decreased cardiac output, and arterial hypotension. It has also been shown that IL-1 activates a myocardial L-arginine-nitric oxide pathway that raises myocardial cyclic GMP and induces twitch abbreviation (15). Our
Present results further showed that intravenous administration of IL-1β decreased stroke volume, cardiac output, and mean arterial pressure in normothermic control animals. These observations tend to indicate that a selective decline in stroke volume or ventricular depolarization resulting from increased plasma levels of IL-1 may be an important mechanism signaling arterial hypotension or circulatory failure in rat heatstroke.

IL-1 has been implicated in the control of responses to systemic disease and injury and activation of fever, neuroendocrine, immune, and behavioral responses (27). Pretreatment with IL-1ra has also been shown to block the thermogenic, anorexic, and behavioral effects of recombinant human IL-1β (17). Both our previous (22–24, 29) and present results further show that some cardiovascular effects of heatstroke such as intracra-

Table 4. Plasma concentrations of IL-1 in control rats without heat exposure and rats with 70-min heat exposure

<table>
<thead>
<tr>
<th>Groups</th>
<th>IL-1 Concentration, U/ml</th>
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<tbody>
<tr>
<td>Control rats</td>
<td>0.92 ± 0.03</td>
</tr>
<tr>
<td>Rats with 70-min heat exposure</td>
<td>11.87 ± 0.39*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 8 rats for each group. *P < 0.05, Significance of difference from control value (group without heat exposure), Student’s t-test.
In summary, the results showed that 1) heatstroke was accompanied by increased plasma levels of IL-1; 2) animals injected with IL-1ra at the time of heatstroke induction were protected from some of the cardiovascular effects of heatstroke such as depressed ventricular depolarization, decreased stroke volume, and arterial hypotension; and 3) the hemodynamic changes associated with heatstroke could be mimicked by IL-1 injection. The data suggest that heatstroke stimulates synthesis and release of IL-1 in the plasma, depresses ventricular depolarization and stroke volume, and results in arterial hypotension.

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REFERENCES