Opioid peptides mediate heat stress-induced immunosuppression during pregnancy

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Nakamura, Hiroyuki, Hirofumi Nagase, Masami Yoshida, Keiki Ogino, Toshio Seto, Kotaro Hatta, and Ichioy Matzuaki. Opioid peptides mediate heat stress-induced immunosuppression during pregnancy. Am. J. Physiol. 274 (Regulatory Integrative Comp. Physiol. 43): R672–R676, 1998.—To clarify the involvement of the opioid system in enhanced immunosuppression induced by heat stress during pregnancy, we examined the effects of heat exposure and intraperitoneal administration of opioid receptor antagonist naloxone on β-endorphin (β-EP) in blood, pituitary lobes, and placenta as well as splenic natural killer cell activity (NKCA) and placental steroids in pregnant rats at 15–16 days gestation. Two-way analysis of variance revealed significant increases in blood β-EP induced by heat and naloxone and a significant interaction between heat and naloxone on blood β-EP and progesterone (P). Whereas heat reduced NKCA, intraperitoneal administration of naloxone reversed it. Significant increases in blood and placental β-EP induced by both heat and naloxone administration and a significant interaction on blood and placental β-EP was observed. These results suggest that immunosuppression produced by heat stress during pregnancy is mediated by the opioid system. A positive correlation between β-EP in blood and placenta during heat and naloxone administration suggests that increased placental β-EP during heat results in hypersecretion of β-EP into blood. P increased by heat during pregnancy may be involved in the immunosuppression.

β-endorphin; natural killer cell activity; pituitary; placenta; progesterone

PREGNANCY PRODUCES adaptive modifications in the homeostasis of the maternal immune system in the survival of the fetoplacental graft (4, 12). Natural killer (NK) cells act early in the immune response before specificity can be generated. They mediate first-line defense by direct cytotoxicity against various types of target cells without apparent prior immunization (34). Heat stress during early or mild gestation pregnancy results in a high incidence of embryo mortality (2, 33). Although we have previously demonstrated enhanced immunosuppression produced by heat stress during pregnancy (18), the neuroendocrine mechanisms in the immunosuppression for pregnant mammals, including humans, exposed to heat stress remain to be elucidated.

Since the immunoassay detection of opiate peptide β-endorphin (β-EP) in placental extracts in 1978 (17), there has been considerable evidence showing the active presence of opiate receptors in human placental villous tissues (1, 35). Genomic and cDNA clones for opioid receptors exist for several animal species, including mouse, rat, guinea pig, and human (14). Human maternal plasma concentration of β-EP is elevated during pregnancy (20). Although the endogenous opioid is involved in stress-induced immunosuppression (28), the effect of pregnancy on immunosuppression during stress is unknown. Placental steroids such as estrogens and progesterone (P) exert a positive effect on the β-EP content in the pituitary lobes (22). To examine the involvement of the opioid system in enhanced immunosuppression induced by heat stress during pregnancy, we examined the effects of heat exposure and intraperitoneal administration of opioid receptor antagonist naloxone on β-EP in blood, pituitary lobes, and placenta as well as splenic NK cell activity (NKCA) and placental steroids in pregnant rats.

MATERIALS AND METHODS

Preparation of virgin and pregnant rats for study. Twenty-four Wistar rats at 15–16 days gestation, weighing 270 ± 4.69 g (means ± SE) were studied. For breeding, a male rat was placed in a cage with two females. The environment was controlled in all cases (23 ± 2°C, 50% humidity), with alternating cycles of 12-h light (8 AM–8 PM) and 12-h dark. The onset of pregnancy was determined using vaginal smears. All animals had access to commercial food and tap water ad libitum. The rats were fasted, but given water in the 24 h before the experiment and deprived of food and drink throughout the experiment. This study was approved by the Ethics Committee on Animal Experimentation of Kanazawa University, Takara-machi Campus. In all cases the experimental protocol began at 11 AM. Twenty-four pregnant rats were divided into the following four groups: six rats with intraperitoneal administration of saline, but not exposed to heat; six rats with intraperitoneal administration of saline before heat; six rats with intraperitoneal administration of naloxone, but not exposed to heat; and six rats with intraperitoneal administration of naloxone before heat.

Intraperitoneal administration of naloxone. Naloxone HCl (Sigma, St Louis, MO) was administered intraperitoneally at a dose of 0.2 ml of 10 mg/ml solution in 0.9% saline 30 min before heat exposure. Twelve pregnant rats received naloxone, and 12 received 0.2 ml of the saline alone. The intraperitoneal administration dose of naloxone (2 mg/rat) is known to reverse the effect of the opioid system on immune changes in rats (13, 24).

Exposure to heat stress. The use of a microwave system is ideal for heat exposure because it allows for the administration of an exact quantity of energy (9). The microwave exposure device, described previously (11), was equipped with a magnetron of 2,450 MHz as the source of energy and had an isolator to vary the energy from the magnetron induced by reflection from the applicator (350 × 470 × 455 mm). Twelve
pregnant rats (6 rats subjected to saline and 6 rats to naloxone) were put into a semicylindrical acrylic plastic holder (thickness, 5 mm; inside diameter, 60 mm; length, 170 mm) and were exposed to microwaves at 10 mW/cm² incident power density at 2,450 MHz for 90 min. The sham-exposed rats (6 rats subjected to saline and 6 rats to naloxone) were treated in an identical manner, except that the microwave generator was not turned on. During exposure, the environment of the exposure facility was maintained at 21–23°C and 50–60% humidity.

Measurements of blood corticosterone, β-EP, estradiol, and P. Blood samples were collected by decapitation of rats immediately after the end of the protocol. Plasma was immediately prepared by transfer of samples to cooled conical centrifuge tubes containing 0.1 mM EDTA followed by centrifugation. The plasma was frozen at −80°C until analysis. Corticosterone (CS) was measured by the fluorometric method of Silber et al. (30). β-EP was measured by the radiommunoassay (RIA) described by Yoshimi et al. (39). In this method, highly purified β-EP was labeled with Na ¹²⁵I using chloramine T. The purification of labeled β-EP was performed on a carboxymethyl cellulose column. The antisera against β-EP showed negligible cross-reactivity with other fragments of β-lipotropin such as α-melanocyte-stimulating hormone and ACTH. Estradiol (E₂) and P were analyzed by RIA using the tube solid-phase method of Ratcliffe et al. (25). The intra- and interassay coefficients of variation were 8.0 and 12.5% for CS, 7.0 and 11.0% for β-EP, 7.5 and 10.6% for E₂, and 5.4 and 7.6% for P, respectively. The sensitivity of the assays for CS, β-EP, E₂, and P were 5 ng/tube and 3, 2.5, and 1.1 pg/tube, respectively.

Splenic NKCA. To measure splenic NKCA, the spleen was surgically excised and dissociated into a single-cell suspension. The splenocytes were suspended in 40 ml phosphate-buffered saline (PBS) and centrifuged in 50-ml tubes at 400 rev/min at 4°C for 20 min. The sham-exposed rats (6 rats subjected to saline and 6 rats to naloxone) were treated in an identical manner, except that the microwave generator was not turned on. During exposure, the environment of the exposure facility was maintained at 21–23°C and 50–60% humidity.

Percent cytotoxicity was calculated at each E/T, and these values were converted to lytic units at 30% (LU₃₀) according to the method of Pross et al. (23). The intra- and interassay coefficients of variance for LU₃₀ as a measure of NKCA were 7.5 and 18.2%, respectively.

RESULTS

Blood CS, β-EP, E₂, and P in heat-exposed or nonexposed pregnant rats with intraperitoneal administration with saline or naloxone are shown in Table 1. Two-way ANOVA revealed that heat stress significantly increased CS and β-EP and decreased E₂, independent of naloxone administration. The ANOVA showed that naloxone administration significantly increased β-EP. There were significant interactions between heat and naloxone administration on β-EP and P. Effects of heat stress and intraperitoneal administration of naloxone on splenic NKCA in pregnant rats are demonstrated in Fig. 1. Whereas heat reduced NKCA, intraperitoneal administration of naloxone reversed it. There was a significant interactive effect on NKCA. We could observe significant increases in placental β-EP induced by both heat and naloxone administration and a significant interaction on it.

Heat was found to increase β-EP in AP, NIL, and placenta significantly. Intraperitoneal administration of naloxone significantly increased β-EP in placenta. A significant interaction on β-EP in placenta was observed (Table 2).

DISCUSSION

Functional networks among nervous, endocrine, and immune systems are now interpreted as a neuroimmune-endocrine function (29). Corticotropin-releasing hormone (CRH; 3, 36) and opiate β-EP (16, 37) play roles in modulating neuroendocrine and immune systems as
neurotransmitters. In agreement with a previous study (18), heat reduced NKCA in pregnant rats. Furthermore, we observed a promoting effect of naloxone administration as well as an interactive effect between heat and naloxone on NKCA. These results suggest that naloxone administration antagonizes immunosuppression induced by heat. Therefore, immunosuppression after heat stress during pregnancy seems to be mediated by the opioid system.

Gel filtration chromatography for β-EP in placental and pituitary extracts has shown that placental β-EP is not involved in analgesia induced by opioid-dependent stress, but plays a paracrine and autocrine role during pregnancy (5). Although the elevation in β-EP induced by heat stress was seen in blood as well as pituitary and placenta, the interactive effect between heat and naloxone on β-EP was seen only in blood and placenta. This result implies that naloxone administration increases β-EP in blood and placenta only in heat-exposed rats, supporting the presence of opioid receptors in the placenta, which was shown by many researchers (1, 35). Simultaneously, our data may provide evidence for the involvement of the placental opioid system in heat stress in a paracrine and autocrine fashion. Falconer et al. (8) have indicated that uteroplacental β-EP secretes into the maternal circulation in response to hyperglycemic stress. Blockade of the placental opioid system by naloxone during heat stress appears to increase placental β-EP, subsequently resulting in hypersecretion of β-EP into blood, which was indicated by increased blood β-EP in pregnant rats receiving naloxone before heat. Because naloxone administration increased β-EP in the placenta, but not in the pituitary of pregnant rats, this appears to support the assumption of Chan and Smith (5) that placental β-EP is not involved in systemic immunosuppression. The physiological role of placental β-EP may be different from that of pituitary β-EP. Several types of opioid receptors have been implicated in the immunosuppression (6, 10, 21), but the type of receptor involved in stress-induced immunosuppression is not clear. Because naloxone is a nonselective opioid receptor antagonist (10, 21), further studies should be designed for determination of the types of opioid receptors involved in heat stress-induced immunosuppression during pregnancy.

Activation of the stress response inhibits the hypothalamic-pituitary-gonadal axis at multiple levels (27, 38). CRH suppresses secretion of luteinizing hormone-releasing hormone in the hypothalamic arcuate nucleus either directly or indirectly via the stimulation of β-EP or corticosteroids (7, 19). In the present study, however, heat stress increased P and naloxone administration reversed it. Because progesterone-induced blocking functions of corticosteroid receptors have been observed (15, 43), it is possible that naloxone administration eliminated the blocking functions of corticosteroid receptors, resulting in suppression of CRH. Further experiments are needed to confirm this hypothesis.

### Table 1. Effects of heat stress and intraperitoneal administration of naloxone before stress on blood indicators in pregnant rats

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Treatment</th>
<th>Number of Rats Examined</th>
<th>CS, ng/ml</th>
<th>β-EP, pg/ml</th>
<th>E2, pg/ml</th>
<th>P, pg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>No heat</td>
<td>Saline</td>
<td>6</td>
<td>282 ± 28.4</td>
<td>130 ± 8.42</td>
<td>64.6 ± 5.95</td>
<td>46.0 ± 2.61</td>
</tr>
<tr>
<td>No heat</td>
<td>Naloxone</td>
<td>6</td>
<td>344 ± 48.0</td>
<td>124 ± 6.82</td>
<td>58.9 ± 9.89</td>
<td>50.4 ± 6.81</td>
</tr>
<tr>
<td>Heat</td>
<td>Saline</td>
<td>6</td>
<td>455 ± 39.1</td>
<td>161 ± 9.41</td>
<td>44.0 ± 3.95</td>
<td>61.6 ± 3.69</td>
</tr>
<tr>
<td>Heat</td>
<td>Naloxone</td>
<td>6</td>
<td>476 ± 36.7</td>
<td>210 ± 15.3</td>
<td>39.2 ± 2.32</td>
<td>44.8 ± 4.95</td>
</tr>
</tbody>
</table>

Values are means ± SE. Statistical analysis of difference was performed by 2-way ANOVA. Significant main effects of heat on corticosterone (CS) F (1,20) = 18.8, P < 0.001; β-endorphin (β-EP) F (1,20) = 37.1, P < 0.001; and estradiol (E2) F (1,20) = 12.7, P < 0.01 and naloxone on β-EP F (1,20) = 4.99, P < 0.05 and interactive effects on β-EP F (1,20) = 8.58, P < 0.01 and progesterone (P) F (1,20) = 5.92, P < 0.05.

### Table 2. Effects of heat stress and intraperitoneal administration of naloxone before heat stress in pregnant rats

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Treatment</th>
<th>Number of Rats Examined</th>
<th>β-Endorphin Concentration, ng/mg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Anterior pituitary lobe</td>
</tr>
<tr>
<td>No heat</td>
<td>Saline</td>
<td>6</td>
<td>525 ± 47.4</td>
</tr>
<tr>
<td>No heat</td>
<td>Naloxone</td>
<td>6</td>
<td>537 ± 41.7</td>
</tr>
<tr>
<td>Heat</td>
<td>Saline</td>
<td>6</td>
<td>709 ± 48.3</td>
</tr>
<tr>
<td>Heat</td>
<td>Naloxone</td>
<td>6</td>
<td>721 ± 59.7</td>
</tr>
</tbody>
</table>

Values are means ± SE. Statistical analysis of difference was performed by 2-way ANOVA. Significant main effects of heat on β-EP in anterior pituitary lobe F (1,20) = 14.7, P < 0.01; neurointermediate pituitary lobe F (1,20) = 14.7, P < 0.01; and placenta F (1,20) = 20.1, P < 0.001 and naloxone on β-EP in placenta F (1,20) = 11.3, P < 0.01 and interactive effect on β-EP in placenta F (1,20) = 5.87, P < 0.05.

Fig. 1. Effects of heat stress and intraperitoneal administration of naloxone on splenic natural killer cell activity (NKCA) in pregnant rats. Values represent means ± SE. Statistical analysis of difference was performed by 2-way analysis of variance. Significant main effect of heat [F(1,20) = 6.67, P < 0.05] and naloxone [F(1,20) = 6.28, P < 0.05] and interactive effect on NKCA [F(1,20) = 9.46, P < 0.01], LU30, Lytic units at 30%.
tor administered in vivo significantly prevented the high rates of resorption in mice treated with antiprogestrone, progesterone-mediated suppression of lymphocyte toxicity plays a significant role in the maintenance of pregnancy (32). Taken together with our results showing that effects of naloxone administration on both P and NKCA were seen only in rats exposed to heat, activated placental hormones including P may be involved in the immunosuppression induced by heat stress during pregnancy. However, the effect of naloxone on E2 in rats with heat was not different from that without heat. On the basis of the fact that stress suppresses placental functions directly or via the opioid systems (7, 19), clarification of the involvement of increased P during heat during pregnancy should be the focus of future work.

Perspectives

The present results regarding naloxone administration with heat in pregnant rats indicate that the immunosuppression produced by heat stress during pregnancy is mediated by the opioid system. Increased EP in blood and placenta by naloxone administration only in heat-exposed rats suggests that blockade of the placental opioid system during heat increases placental β-EP in a paracrine and autocrine fashion, subsequently resulting in hypersecretion of β-EP into blood. Interestingly, the neurochemical relationship between CRH and the opioid-containing systems in the hypothalamic-pituitary axis also exists in the placenta. In placenta cells in culture, synthetic CRH stimulates the release of β-EP in a dose-dependent manner (31). The physiological significance of the placental opioid system, especially in relation to placental CRH should be clarified by future studies. We measured the alterations of β-EP in the pituitary and placenta in pregnant rats exposed to heat. Further direct evidence for the involvement of opioid systems in heat-stress-induced immunosuppression during pregnancy would be obtained by examination of the expression of opioid receptor mRNA in those tissues.

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