Stress-induced alterations of immunity in hypophysectomized rats

(behavior/T cell/natural killer cell/ pituitary)

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ABSTRACT Stress-induced suppression of mitogen-induced lymphocyte proliferation was demonstrated in hypophysectomized rats. Stress effects on the numbers of peripheral blood lymphocytes and lymphocyte subsets and on splenic natural killer cell activity require the presence of pituitary. A pituitary-dependent restraining influence on stress-induced alteration of immunity is described. These results indicate that stress-induced modulation of immunity is complex and includes a range of enhancing and inhibitory mechanisms.

A wide range of stressors alter immunity in animals and humans. The specific mechanisms that mediate these stress effects have not been fully elucidated. The endocrine system is highly responsive to stress and neuroendocrine activity and, through the hypothalamic-pituitary axis, may be involved in stress-induced alterations of the immune system. The secretion of corticosteroids has long been considered the mechanism of stress-induced modulation of immunity. We have shown stress-induced suppression of lymphocyte proliferation in adrenalectomized rats, indicating that corticosteroid-independent mechanisms participate in stress-related modulation of immunity (1). Because a variety of other hormones under pituitary control have been associated with immunoregulatory processes (2), we investigated the role of the pituitary in mediating stress-induced alterations of immunity, examining the effects of a stressor on immune function in hypophysectomized rats.

MATERIALS AND METHODS

A total of 96 Sprague-Dawley male rats (Zivic-Miller, Pittsburgh) and a "3 × 2" experimental design (16 rats per group) were used. The third groups consisted of nonoperated, sham-hypophysectomized, and hypophysectomized rats. The two treatments, home cage controls and stressed animals, were similar to those used in our previous studies (2, 3) with the level of shock 0.6 mA for the first 6 hr, 1.2 mA for the second 6 hr, and 1.8 mA for the final 6 hr. In our previous studies, we used the Wistar-Furth strain of rat and a stress schedule that had been shown to elicit a classic stress response (1, 3). In the present study, we used the Sprague-Dawley strain of rat, since this animal is harder and thereby better suited for experiments requiring hypophysectomy. In a series of preliminary studies, we titrated the intensity of the electric shock to the minimum level that would allow the differentiation between home cage and stressed animals in terms of the phytohemagglutinin (PHA) response.

Hypophysectomy and sham hypophysectomy were performed by standard techniques (Zivic-Miller) Two to 3 weeks were allowed for recovery from operative procedures. All animals were given free access to food, tap water, and a saline solution, and were maintained in a quiet room with the temperature carefully regulated at 70°F (21.1°C). All experiments were conducted at the same time of day.

After the shock period, the rats were removed from the apparatus or home cages, anesthetized with ether, and exsanguinated by heart puncture within 3 min using preservative-free heparin. Portions of blood from each animal were used for each of the in vitro studies. Absence of the pituitary tissues was confirmed by necropsy. After exsanguination, each animal was perfused with heparinized saline and the spleen and brain were removed. Total leukocyte and differential counts were performed by standard techniques. T cells (clone W3/13L), B cells, T-helper cells (clone W3/25), and T-suppressor cells (clone OX-8-nonhelper) were enumerated by using monoclonal antibodies and fluorescent microscopy. The monoclonal antibodies used in enumerating lymphocyte cell type were purchased from Accurate Chemicals (Westbury, NY). Mitogen-induced lymphocyte stimulation was carried out as described by using a dose response to both PHA and Con A. Micromethods developed by Keller et al. (4) were used to assess PHA- (Wellcome) and Con A- (Cibrochem-Behring) induced stimulation of lymphocytes isolated from rat peripheral blood. Stimulation of splenic lymphocytes was carried out according to the method of Sell et al. (5) and Hem (6). All cultures were prepared in triplicate. Dose responses to PHA and Con A were used (PHA: 0.4, 0.8, and 1.6 µg per culture; Con A: 0.25, 0.5, and 1.0 µg per culture). For both the isolated peripheral blood and spleen cell assays we used 5 × 10⁴ lymphocytes per culture. The lymphocyte stimulation data in the figures and the data used for the statistical analysis are expressed as cpm in the stimulated cultures minus cpm in the corresponding unstimulated cultures (Δcpm). The response at the optimal dose was used as the dependent variable for the statistical analysis.

Natural killer (NK) cell activity was assessed in the 4-hr ⁵¹Cr release assay utilizing YAC1 target cells as described (7). Five effector/target ratios were used to assess both peripheral blood and splenic NK cell function.

Brain tissue was extracted for neuroendocrine assessments (8). Plasma corticosterone and corticotropin (ACTH) and whole brain corticotropin-releasing factor (CRF) and β-endorphin concentrations were measured by radioimmunoassay as described (9-11).

RESULTS AND DISCUSSION

The body weights of the hypophysectomized animals (Table 1) were significantly lower than those of the two control groups, consistent with a lack of pituitary-released growth hormone.

Abbreviations: PHA, phytohemagglutinin; NK cells, natural killer cells; ACTH, corticotropin; CRF, corticotropin-releasing factor; ANOVA, analysis of variance.

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Plasma concentrations of both ACTH and corticosterone were increased in the stressed groups with pituitaries and were below detectable levels in the animals without pituitaries (Fig. 1a and b). In the hypophysectomized animals, there was an increase in whole brain CRF and a decrease in whole brain β-endorphin in both treatment conditions (Fig. 1c and d). The concentrations of whole brain CRF and β-endorphin as well as plasma ACTH and corticosterone levels were similar in the nonoperated and sham-hypophysectomized groups, indicating that the 2-week postoperative recovery period was sufficient to allow return to baseline levels and that the operative procedures did not have permanent effects on these stress-related peptides and hormones. Completeness of hypophysectomy was confirmed by the absence of detectable levels of plasma ACTH and corticosterone in the hypophysectomized animals. The lack of stress effects on whole brain CRF or β-endorphin concentrations is consistent with previous observations (12, 13) that only CRF- and β-endorphin-containing neurons within the hypothalamic region are affected by stress and suggest that neurons in other brain regions containing these neuropeptides are not directly associated with stress-related immune alterations.

In both the nonoperated and sham-hypophysectomized groups, there was a stress-induced lymphopenia in peripheral blood (Table 2) (t = 3.03; df = 89; P < 0.01). In addition, there was a stress-related decrease in the number of T lymphocytes and T-helper lymphocytes (T cells, t = 2.45 and P < 0.02; T-helper cells, t = 2.64 and P < 0.02) but not in the number of T-suppressor lymphocytes. The number of B lymphocytes was not altered by the stressful condition. In the hypophysectomized animals, no stress-related changes were found in the absolute number of peripheral blood lymphocytes or lymphocyte subsets.

We previously demonstrated a stress-induced decrease in

![Graph](image)
the number of peripheral blood lymphocytes in the rat that is abrogated by adrenalectomy (1). The present study extends these observations, demonstrating that the stress-induced lymphopenia in the rat is selective for T cells, specifically T-helper cells. In addition, the stress-induced lymphopenia is pituitary dependent and is found concurrent with increased levels of plasma ACTH and corticosterone, consistent with the observation that the number of circulating immunocompetent cells in response to a stressor is regulated by the hypothalamic–pituitary axis. The stress-related decrease in lymphocyte numbers from the peripheral blood may be related to vascular margination or migration into the interstitial compartment, the lymphatics, or lymph nodes.

The present study also examined the effects of the stressor on the number of splenic lymphocytes, representing another major immunologic compartment. In the nonoperated and sham-hypophysectomized animals, the stressor was associated with a significant decrease in the numbers of splenic lymphocytes, T and B cells, and T-cell subsets (Table 3; \( P < 0.01 \) in all cases). There were no significant stress effects seen in the hypophysectomized group for any of the splenic lymphocyte cell types. Hypophysectomy itself, however, resulted in decreased numbers of splenic lymphocytes so that it may be difficult to detect stress effects. Furthermore, the stressor was associated with a decrease in spleen weight in all groups. The data were therefore also analyzed as cells per g of spleen. No stress effects were found in any of the groups, suggesting that the stress effects on the number of splenic lymphocytes may be related to compartment size.

As shown in Fig. 2A, the stressful condition suppressed PHA-induced stimulation of peripheral blood lymphocytes in the hypophysectomized animals as well as in the control groups, with the magnitude of suppression of the response greatest in the hypophysectomized rats. ANOVA revealed a highly significant treatment effect \( (F = 38.4; \text{df} = 1, 90; P < 0.001) \) and group effect \( (F = 52.1; \text{df} = 1, 90; P < 0.001) \) and treatment by group interaction \( (F = 13.6; \text{df} = 1, 90; P < 0.001) \). Single degree of freedom contrasts revealed that there was a stress effect in the control groups \( (t_0 = 2.39; P < 0.05) \) and in the hypophysectomized animals, with the lymphocyte responses of the stressed hypophysectomized animals significantly lower than the nonstressed hypophysectomized rats \( (t_0 = 5.91; P < 0.001) \). The magnitude of the stress-induced suppression of peripheral blood lymphocyte function in the hypophysectomized animals is significantly greater than in animals with pituitaries \( (t_0 = 7.55; P < 0.001) \).

These findings demonstrate that factors not of pituitary origin mediate the stress-induced suppression of peripheral blood lymphocyte proliferation. In addition to the hypothalamic pituitary axis, the autonomic nervous system is another major stress-activated system and stress-induced modulation of lymphocyte function may be related to neurotransmitter alterations. Utilizing a stressor similar to that used in the present study, Weiss and Simson (14) found marked depletion of norepinephrine in various regions of the rat brain including the hypothalamus and locus ceruleus. We suggest that the present finding of a pituitary-independent stress-induced suppression of peripheral blood lymphocyte proliferation is related to central and peripheral catecholamine systems, which have been shown to regulate immune processes (15, 16). It may well be that the final neural link to the immune system, through which this pituitary-independent effect is mediated, involves the postganglionic autonomic nerve fibers that have been shown to terminate in the parenchyma of lymphoid organs in close association with both T lymphocytes and macrophages (17).

As described above, another major finding of the present study was that the magnitude of the suppression of lymphocyte activity in response to the stressor was far greater in the hypophysectomized rats than in the control animals. These data demonstrate that pituitary processes are involved in counteracting stress-induced immunosuppressive mechanisms. While the specific pituitary-dependent mitigating or compensating processes are not known, these findings suggest that there is a regulatory network of hormonal and nonhormonal systems involved in the maintenance of immunologic capacity after exposure to stressors. These findings suggest additional avenues for research into the relationship among stressors, immunity, and health.

In contrast to the findings with peripheral blood lymphocytes, there were no systematic stress effects on splenic lymphocyte stimulation of PHA (Fig. 2B). ANOVA revealed

### Table 2. Absolute number of peripheral blood lymphocytes and lymphocyte subsets

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Nonoperated</th>
<th></th>
<th>Sham hypophysectomy</th>
<th></th>
<th>Hypophysectomy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Home cage control</td>
<td>Shock</td>
<td>Home cage control</td>
<td>Shock</td>
<td>Home cage control</td>
<td>Shock</td>
</tr>
<tr>
<td>Total lymphocytes</td>
<td>6.71 ± 0.54</td>
<td>5.69 ± 0.29</td>
<td>7.00 ± 0.48</td>
<td>4.48 ± 0.52</td>
<td>7.25 ± 0.61</td>
<td>7.31 ± 0.92</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>3.90 ± 0.42</td>
<td>2.77 ± 0.52</td>
<td>4.02 ± 0.65</td>
<td>2.55 ± 0.53</td>
<td>4.06 ± 0.75</td>
<td>3.75 ± 0.54</td>
</tr>
<tr>
<td>B helper cells</td>
<td>1.38 ± 0.31</td>
<td>1.43 ± 0.31</td>
<td>1.82 ± 0.45</td>
<td>1.22 ± 0.36</td>
<td>2.24 ± 0.65</td>
<td>1.72 ± 0.18</td>
</tr>
<tr>
<td>T-suppressor cells</td>
<td>2.85 ± 0.55</td>
<td>1.72 ± 0.21</td>
<td>2.51 ± 0.56</td>
<td>1.40 ± 0.35</td>
<td>2.68 ± 0.64</td>
<td>2.64 ± 0.74</td>
</tr>
<tr>
<td></td>
<td>2.50 ± 0.62</td>
<td>1.68 ± 0.41</td>
<td>1.49 ± 0.35</td>
<td>1.03 ± 0.23</td>
<td>2.34 ± 0.66</td>
<td>2.08 ± 0.52</td>
</tr>
</tbody>
</table>

The data are expressed as \( 10^4 \) cells per ml (mean ± SEM).

### Table 3. Absolute number of splenic lymphocytes and splenic lymphocyte subsets

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Control*</th>
<th></th>
<th>Hypophysectomy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Home cage control</td>
<td>Shock</td>
<td>Home cage control</td>
<td>Shock</td>
</tr>
<tr>
<td>Total lymphocytes</td>
<td>50.6 ± 4.2</td>
<td>31.0 ± 2.0</td>
<td>21.9 ± 2.4</td>
<td>15.3 ± 2.2</td>
</tr>
<tr>
<td>T lymphocytes</td>
<td>41.9 ± 4.2</td>
<td>16.0 ± 1.6</td>
<td>4.3 ± 0.6</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>B lymphocytes</td>
<td>30.4 ± 3.3</td>
<td>13.4 ± 1.4</td>
<td>3.9 ± 0.8</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>T-helper cells</td>
<td>24.9 ± 3.3</td>
<td>11.3 ± 2.0</td>
<td>3.5 ± 0.5</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>T-suppressor cells</td>
<td>21.2 ± 2.8</td>
<td>9.5 ± 1.7</td>
<td>2.9 ± 0.6</td>
<td>1.6 ± 0.3</td>
</tr>
</tbody>
</table>

The data are expressed as \( 10^7 \) cells per spleen (mean ± SEM).

*Mean of the nonoperated and sham hypophysectomy.

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\(^{1}\)Weight of spleens in g (mean ± SEM): nonoperated: home cage control, 0.753 ± 0.037 g; shock, 0.577 ± 0.039 g; sham hypophysectomy: home cage control, 0.804 ± 0.036 g; shock, 0.507 ± 0.040 g; hypophysectomy: home cage control, 0.208 ± 0.014 g; shock, 0.162 ± 0.016 g.
no significant treatment effect ($F = 0.19; \text{df} = 1, 88; P$, not significant), group effect ($F = 2.84; \text{df} = 2, 88; P$, not significant), or treatment by group interaction ($F = 0.05; \text{df} = 2, 88; P$, not significant). The lack of a stress effect on the

stimulation of splenic lymphocytes in contrast to peripheral blood lymphocytes may reflect differences in the various compartments of the immune system, each with its own microenvironment and subject to specific modulators and

FIG. 2. Stimulation of peripheral blood lymphocytes (A) and spleen cells (B) by PHA for each of the three operative groups and two treatment procedures. Data (mean ± SEM) are represented as Δcpm. —, Home cage control; ---, shock.

FIG. 3. NK cell activity (mean ± SEM) of peripheral blood (A) and spleen (B) against YAC1 cells for each of the three operative groups and two treatment procedures. —, Home cage control; ---, shock.
regulators. Splenic catecholamines, for example, have been
known to induce a migration of lymphocytes from spleen to
the peripheral blood (18, 19) and, thereby, may produce a
selective redistribution of cell types resulting in suppressed
peripheral blood mitogen response without an evident stress
effect in the spleen."** These findings suggest that the
assessment of the consequences of stress for the organism
must consider effects on each of the major immunologic
compartments.

The effect of the stressful condition on peripheral blood
NK cell function is shown in Fig. 3A. ANOVA revealed no
significant overall stress effects (F = 0.03; df = 1, 90; P, not
significant), a significant group effect (F = 12.07; df = 1, 90;
P < 0.001), and no significant treatment by group interaction
(F = 3.55; df = 1, 90; P, not significant). These findings
indicate that circulating NK cells are not responsive to the
stressor used in this study. The low absolute levels of NK cell
activity in the nonstressed groups may have precluded the
demonstration of stress effects in the peripheral blood.

In contrast to the peripheral blood compartment, the stress-
ful condition suppressed splenic NK cell activity in the
nonoperated and sham-operated group but had no suppress-
effect in the hypophysectomized group (Fig. 3B). ANOVA revealed a highly significant treatment effect (F = 8.30; df = 1, 90; P < 0.01), a highly significant group effect
(F = 7.35; df = 1, 90; P < 0.01), and a trend toward a
treatment by group interaction (F = 3.85; df = 1, 90; P <
0.06). Single degree of freedom contrasts showed that the
stressful condition suppressed the levels of splenic NK cell
activity in the control groups (t0 = 4.14; P < 0.001) but had
no effect on splenic NK cell activity in hypophysectomized animals (t0 = 0.56; P, not significant). The results of the
splenic NK cell assays suggest that stress-induced suppress-
ion of splenic NK cell activity requires the pituitary.
However, since the baseline NK cell activity was lower in
hypophysectomized rats compared with controls (t0 = 3.88;
P < 0.05), further suppression of NK cell activity following
the stressor may have been difficult to detect.

Our findings of a stress-induced pituitary-dependent lymph-
openia, a pituitary-dependent suppression of splenic NK cell
activity, pituitary-independent stress effects on peripheral
blood lymphocyte stimulation, and a pituitary-restrain-
ing influence on the stress-induced suppression of peripheral
blood lymphocyte proliferation indicate that stress-induced
modulation of immunity is complex and involves a range of
mechanisms. Interactions between the nervous system and the

**In both the peripheral blood and splenic lymphocyte stimulation
assays, Con A was also used. The results were the same as with
PHA and the data are not reported.

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