Role of Adrenocorticotropic Hormone in the Activation of Heparin Secretion by Mast Cells in Stressed Rats

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A morphometric analysis of mast cell populations in the subcutaneous tissue and mesentery from rats demonstrated stimulation of heparin secretion by adrenocorticotropic hormone. Thirty minutes after the administration of this hormone to unstressed rats, the functional status of mast cells did not differ from that of such cells from rats stressed by being immobilized for 30 min after receiving physiological saline instead of the hormone. In contrast, the 30-minute immobilization failed to elicit an adequate secretory response from the mast cells of rats in which the release of adrenocorticotropic hormone had been blocked by dexamethasone.

Key Words: heparin; mast cells; ACTH; stress

Processes safeguarding the body against possible thrombosis undoubtedly occupy a central place in adaptive humoral responses to stressors. These processes include, first and foremost, the release of heparin, a direct-acting anticoagulant, into the circulation. Enhanced heparin release from mast cells and heightened anticoagulant potential of the blood during stress have been described [1,6-8].

Since the body's adaptation to stimulants begins at the neuroendocrine level, namely with excitation of the sympathoadrenal and hypothalamus-hypophysis-adrenal cortex systems, it is logical to assume that the excitation of these systems is causally related to the intensity of heparin secretion. Indeed, as our previous studies showed [3], the activation of heparin secretion by mast cells in stress involves the participation of circulating catecholamines, primarily epinephrine. The present investigation was undertaken to elucidate the role played in this activation by a second component of the adaptive system, adrenocorticotropic hormone (ACTH).

MATERIALS AND METHODS

The study was conducted on random-bred male white rats weighing 180-200 g. ACTH (Russian-made) was injected into the jugular vein of test rats in a volume of 1 ml (75 U/kg body weight). Control rats received the same volume of physiological saline. In order to block ACTH secretion, test rats were injected intraperitoneally with dexamethasone (Galenika) twice (at 100 and 200 μg/kg) on the day preceding the day of the test and once (at 100 μg/kg) 1.5 h before the test [4]. The animals were stressed by being immobilized (attached to a small table) for 30 min.

Morphometric analysis of mast cells was carried out in film preparations fixed in buffered formalin solution and stained with a 0.5% toluidine blue solution, pH 4.0 [2]. In these preparations, mast cells with different staining intensities - very dark, dark, light, and very light cells - correlating
with their heparin content were counted. The secretory status of mast cells was evaluated by using a heparin saturation index, defined as the ratio of the sum of very dark and dark cells to that of light and very light cells.

Another indicator which is customarily used in morphometric analyses is the granulolysis index, defined as the ratio of the number of very light (depleted) cells to the total number of cells. This index, however, does not take into account the less depleted (light) cells from which heparin is also released through the lysis of mast cell granules. For this reason, to ensure a more complete assessment of heparin secretion, we deemed it necessary to introduce an "index of total granulolysis" as the ratio of all mast cells secreting heparin at a given time (light plus very light cells) to the total number of counted cells.

The data were treated statistically by Student's t test.

RESULTS

To clarify the role of ACTH in heparin secretion by mast cells, two series of tests were run, the effect of ACTH on heparin secretion in the absence of stress being studied in the first series, and the effect of stress on heparin secretion after blocking ACTH secretion in the second series.

The results of the first test series are summarized in Table 1. In ACTH-injected rats, the condition of the subcutaneous mast cell population 30 min postinjection did not differ from that in animals subjected to immobilization and given saline instead of ACTH: in these two groups, the heparin saturation index was significantly (by 64%) lower than in the intact group, while the total granulolysis index, which characterizes heparin secretion intensity [5], was significantly (1.7-fold) higher (Table 1).

The differences between the ACTH-treated or immobilized and intact groups are clearly illustrated by the quantitative ratios of mast cells differing in heparin levels (ranging from heparin-filled very dark cells to almost completely depleted very light ones) (Fig. 1). As shown in this figure, the mast cell populations of ACTH-treated rats and of rats stressed by immobilization contained 1.8 times more light and very light cells that had released heparin than did the mast cell population from intact rats. This indicates that, in fact, ACTH fully reproduced the effect of the immobilization stress.

In the second test series, ACTH release by mast cells was blocked in rats by repeated dexamethasone injections. After the 30-minute immobilization of these rats, heparin secretion by subcutaneous mast cells was increased, but to a much lesser extent than in the control animals given saline before being stressed (Table 2). As is evidenced by the values of the heparin saturation index, heparin secretion in the stressed rats with blocked ACTH release was of intermediate intensity, being higher than in the stressed controls but lower than in the unstressed controls. The values of the total granulolysis index were also intermediate in the group of stressed rats with blocked ACTH release, its mean value being 0.49±0.02 vs. 0.34±0.02 in the intact controls (Table 1) and 0.57±0.03 in the stressed controls (Table 2).

Dexamethasone itself did not influence heparin secretion by subcutaneous mast cells in the treatment regimen used, and the heparin saturation index in this group was very close to that in the group given saline (Table 2).

### TABLE 1. Effect of ACTH on the Functional Status of Subcutaneous Mast Cells

<table>
<thead>
<tr>
<th>Group</th>
<th>Heparin saturation index</th>
<th>Total granulolysis index</th>
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<tbody>
<tr>
<td>Intact rats (n=9)</td>
<td>1.96±0.20</td>
<td>0.34±0.02</td>
</tr>
<tr>
<td>ACTH–treated rats (n=15)</td>
<td>0.72±0.04*</td>
<td>0.58±0.01*</td>
</tr>
<tr>
<td>Immobilized rats (n=9)</td>
<td>0.69±0.03*</td>
<td>0.57±0.03*</td>
</tr>
</tbody>
</table>

Note. *p<0.01 in comparison with intact rats.