Effects of Cold Stress and Epinephrine on Degranulation of Peritoneal Mast Cells in Rats

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We studied the effects of histamine liberators calcium ionophore A23187 and substance 48/80 on mast cells during cold stress and epinephrine load. Under the effect of both stress factors, ionophore A23187-induced histamine release from mast cells underwent more pronounced changes than that stimulated by substance 48/80. Cold stress and epinephrine load produce different changes in functional activity of Ca^{2+} channels in mast cell membranes.

Key Words: *cold stress; epinephrine shock; mast cells; histamine*

The reaction to extreme environmental factors (hypoxia, cooling, heating, and irradiation) to a great extent depends on neuroregulatory processes, including mobilization of endogenous protective substances histamine, serotonin, catecholamines, and nonprotein thiols [1,5,7]. Mast cells (MC) synthesize and accumulate bioactive substances and then release them by exocytosis [3,4,8]. The mechanisms of exocytosis are well studied, while the specificity of the effect of various adverse factors received little attention. The model of MC degranulation stimulated by liberators is used to study exocytosis. *In vitro* experiments demonstrated the effects of mid-wavelength UV and long-wavelength red lights on stimulated histamine secretion by MC. Mid-wavelength UV inhibits, while red light stimulates histamine release from MC [2]. It is very interesting to study the role of MC and changes in their functions in the organism exposed to various extreme factors. Here we studied *in vivo* effects of cold stress and epinephrine load on exocytosis in rat MC stimulated by liberators acting via different mechanisms.

MATERIALS AND METHODS

Experiments were carried out on purified fraction of peritoneal MC from female Wistar rats. Cold stress was modeled in cold baths (4°C, 5 min) and epinephrine load was produced by subcutaneous injection of 0.1% epinephrine (0.5 ml). The rats were decapitated under chloroform anesthesia immediately or 30 min after cold stress and 15 or 30 min after epinephrine administration. Peritoneal MC were isolated as described elsewhere [10]. The purity of MC fraction was 90-95%.

Immediately after isolation selective histamine liberator substance 48/80 or calcium ionophore A23187 were added to cell suspension in doses of 0.125 and 0.25 μg/ml and the samples were incubated at 37°C for 10 min. The concentration of histamine was measured by the fluorescent method [9] on a Hitachi-850 spectrophotometer. The histamine release was estimated by the ratio of fluorescence in the precipitate and supernatant.

RESULTS

Cold stress modulated degranulation of MC stimulated by ionophore A23187 (Fig. 1). The decrease in histamine release depended on the concentration of ionophore A23187 and the time of observations. Ionophore A23187-stimulated histamine release insignificantly decreased immediately after cold stress, while 30 min after exposure this parameter was suppressed by 15 and 25% (ionophore concentrations 0.125 and 0.25 μg/ml, respectively; Fig. 1, *a*). Cold stress did not change histamine release induced by substance 48/80 (Fig. 1, *b*).

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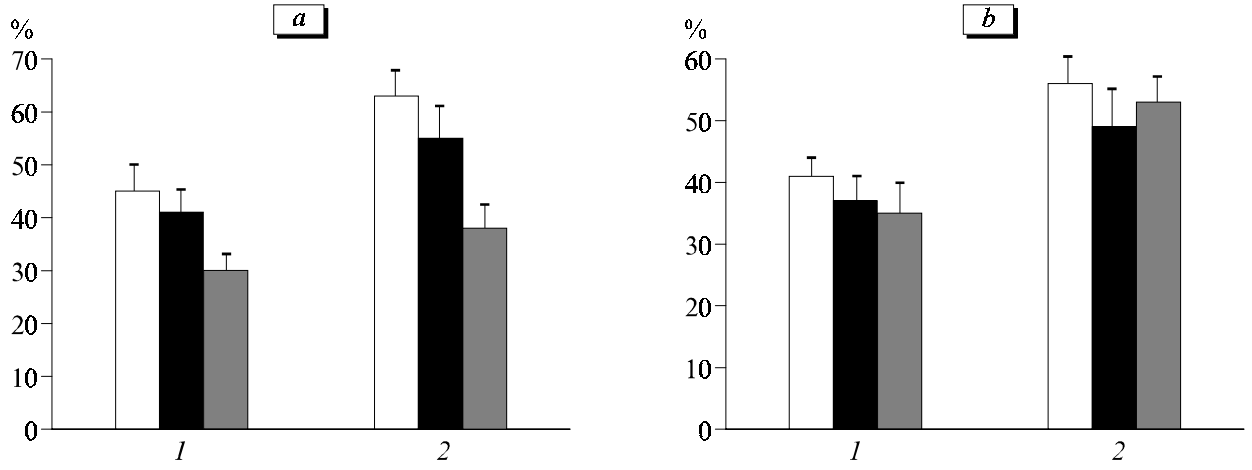

Fig. 1. Effect of cold stress on histamine release from mast cells stimulated by ionophore A23187 (*a*) and substance 48/80 (*b*) in concentrations of 0.125 (*1*) and 0.25 μg/ml (*2*). Light bars: control; dark bars: immediately after cold stress; shaded bars: 30 min after cold stress.

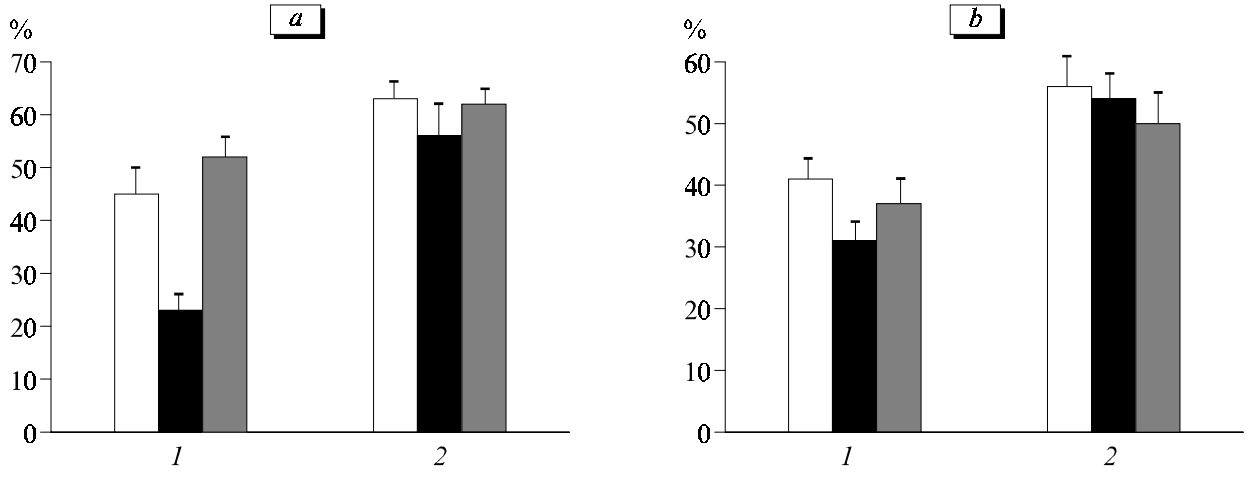

Fig. 2. Effect of epinephrine administration on histamine release from mast cells stimulated by ionophore A23187 (*a*) and substance 48/80 (*b*) in concentrations of 0.125 (*1*) and 0.25 μg/ml (*2*). Light bars: control; dark bars and shaded bars: 15 and 30 min after epinephrine administration, respectively.

Epinephrine also changed MC degranulation (Fig. 2): 15 minutes after epinephrine administration, histamine release induced by calcium ionophore A23187 in a concentration of 0.125 μg/ml decreased 2-fold (Fig. 2, *a*). After stimulation with substance 48/80 degranulation of MC was inhibited to a lesser extent (Fig. 2, *b*). Thirty minutes after injection of epinephrine MC reactions to both histamine liberators did not differ from the control (Fig. 2). General state of rats correlated with these changes: it worsened 15 min after epinephrine administration, and recovered 30 min post-injection.

Our findings indicate that under the effect of both stress factors, histamine release from MC stimulated by ionophore A23187 underwent more pronounced changes than that stimulated by substance 48/80. Cold stress and epinephrine load markedly modulated membrane-associated functions of MC, in particular, liberator-induced secretion of mediators. Since calcium ions play a key role in MC exocytosis [6], these differences are probably related to various mechanisms of Ca^{2+} mobilization by liberating agents: calcium ionophore A23187 induces the entry of exogenous calcium, while substance 48/80 increases intracellular calcium concentration through its release from intracellular stores [6,8]. Therefore, these stress factors probably produce different changes in functional activity of Ca^{2+} channels in MC membranes. The mechanisms underlying functioning of MC under stress conditions require further detailed investigations.

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