Immunoprotective Effects of Prolactin during Stress-Induced Immune Dysfunction

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We showed for the first time that prolactin stimulates the synthesis and release of immunomodulating cytokines and lymphocyte-activating factors (*e.g.*, interleukin-1) by peritoneal macrophages. Prolactin abolished the stress-induced inhibition of proliferation of peripheral blood lymphocytes and increased cell sensitivity to regulatory effects of interleukin-1 in the reaction of lymphocyte blast transformation. These data illustrate the mechanism of immunoprotective activity of prolactin during stress.

Key Words: *prolactin; stress; lymphocyte-activating factors; lymphocyte proliferation*

The development of immune dysfunction during stress is related to disturbances in the interaction between the immune and neuroendocrine systems. This interaction involves a variety of immunomodulating cytokines and hormones, including prolactin [1,2,6]. Prolactin is a peptide hormone of the pituitary gland, which belongs to the family of cytokines and hemopoietic biological substances with regulatory activity (interleukin, IL; somatotropin; growth factors). This substance promotes maturation and differentiation of immune cells [3]. Membrane-bound receptors for prolactin belong to the superfamily of cytokine receptors and are present on various immunocompetent cells. Its physiological effects are realized via the interaction with other cytokines of the "cytokine network" [6,9]. Prolactin increases functional activity of immunocompetent cells and immune system, stimulates the humoral and cellular immune response [4], increases expression of IL-2 receptors on T lymphocytes, activates DNA synthesis, and affects differentiation of T lymphocytes [11]. Prolactin acts as a comitogen and stimulates mitogen-induced proliferation of T lymphocytes [6,14], increases phagocytic activity and chemotaxis of macrophages [12], and potentiates the cytotoxic effect of T killer lymphocytes [10].

Pretreatment with prolactin abolishes stress-induced and glucocorticoid-induced immunosuppression and apoptosis in thymocytes, which is related to immunoprotective activity of this compound [2,4-6]. The mechanisms for this phenomenon were evaluated in *in vitro* experiments. Previous studies showed that early expression and directed differentiation of IL-2 receptors on T lymphocytes depend on the scheme of treatment with prolactin and glucocorticoid hormones [5, 11]. The ratio between the doses of these ligands is of importance for intensive proliferation of lymphocytes under the influence of prolactin [14]. Cellular and molecular studies showed that Stat-5 kinase (signal molecule for prolactin transduction) blocks binding of the glucocorticoid-receptor complex to the specific DNA site and abolishes the immunosuppressive effect of glucocorticoid hormones [8]. Recent studies evaluated the mechanisms for immunomodulating activity of prolactin during stress. Particular attention was given to the functional interaction of prolactin with immunomodulators affecting activity of the immune system [6,9]. These compounds include glucocorticoid hormones and cytokine IL-1 that induce a protective response during stress and are considered as interacting

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elements of the neuroimmunoregulatory chain [1,2]. However, the mechanisms and the role of this interaction in the immunoprotective effect of prolactin during stress remain unclear.

Here we studied the effect of prolactin on stress-induced changes in the immune system, in particular, its effect on humoral immune response, production of lymphocyte-activating factors (LAF) by peritoneal macrophages, and proliferative activity of peripheral blood lymphocytes from animals.

MATERIALS AND METHODS

Experiments were performed on male Wistar rats weighing 180-200 g. The animals were maintained at -20°C for 20 min (cold stress). Human prolactin (Sigma) in doses of 5.25 and 40 ng per 100 g body weight was injected intraperitoneally 20 min before stress. Control rats received an equivalent volume of physiological saline (0.5 ml). In series I the animals were immunized with sheep erythrocytes (1×10^9 cells) immediately after stress. The humoral immune response was studied by the method of direct hemagglutination (plasma antibody titer) on day 5 after immunization.

In series II rat peritoneal macrophages were taken 10 min and 24 h after stress. The intensity of LAF production was estimated by the comitogenic effect of supernatants from incubated cells on proliferation of thymocytes stimulated with lectins in the suboptimal dose [13]. Lipopolysaccharide (LPS) in a dose of 20 μg/ml was used to activate peritoneal macrophages and induce LAF secretion. Peripheral blood lymphocytes were obtained from the abdominal aorta 10 min and 24 h after stress and cultured with concanavalin A (Con A, 0.625 μg/ml) and native rabbit IL-1β (specific activity 2.5×10^4 U/mg protein) to study the comitogenic effect of IL-1 in the reaction of blast transformation. Lymphocyte proliferation was assayed by incorporation of ^{3}H-thymidine into lymphocyte DNA over 1 min.

The results were analyzed by Student's *t* test.

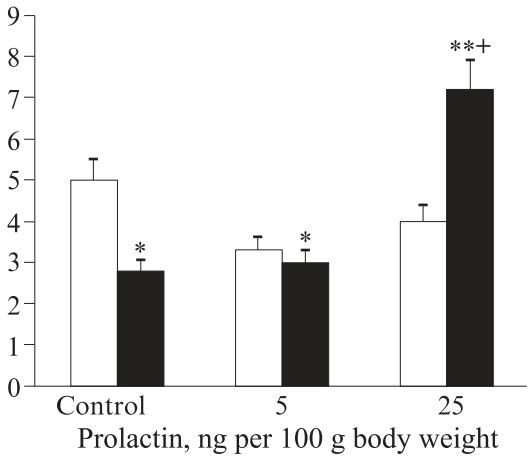

Fig. 1. Humoral immune response in nonstressed (light bars) and stresses animals (dark bars) after prolactin treatment and cold stress. Ordinate: antibody titer in rat plasma, -log 2. $p<0.05$: *compared to nonstressed control animals; **compared to nonstressed animals of the same group; +compared to the control.

RESULTS

Intraperitoneal injection of prolactin in doses of 5 and 25 ng per 100 g body weight had no effect on the humoral immune response to sheep erythrocytes (compared to control immunized animals, Fig. 1). Cold stress was accompanied by suppression of the immune

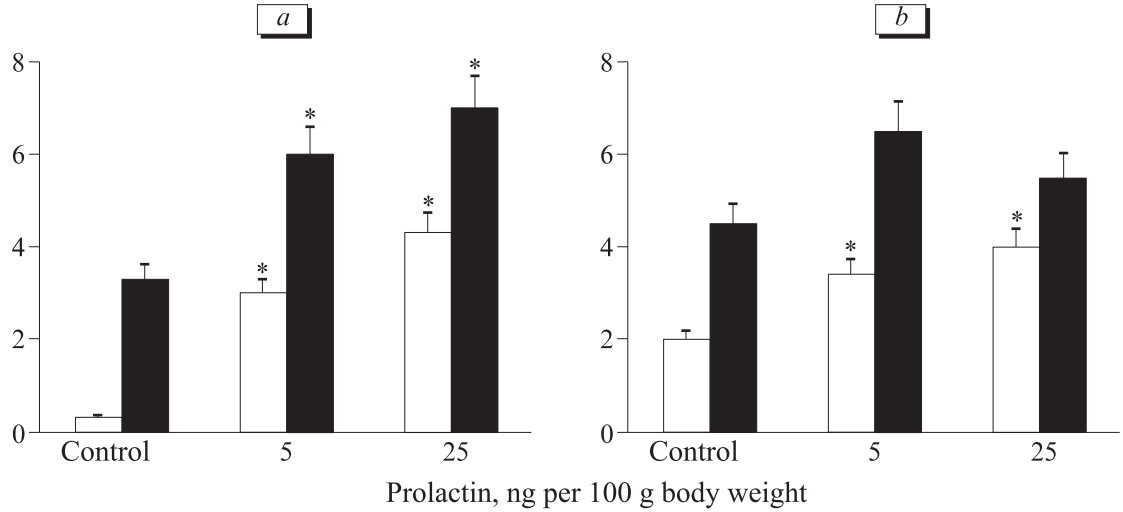

Fig. 2. Production of lymphocyte-activating factors by peritoneal macrophages from nonstressed (*a*) and stresses animals (*b*) receiving intraperitoneal injections of prolactin. Ordinate: activity of the supernatant from peritoneal macrophages, arb. units$\times10^{-3}$. Macrophages nonstimulated (light bars) and stimulated with LPS (dark bars). *$p<0.05$ compared to the control.

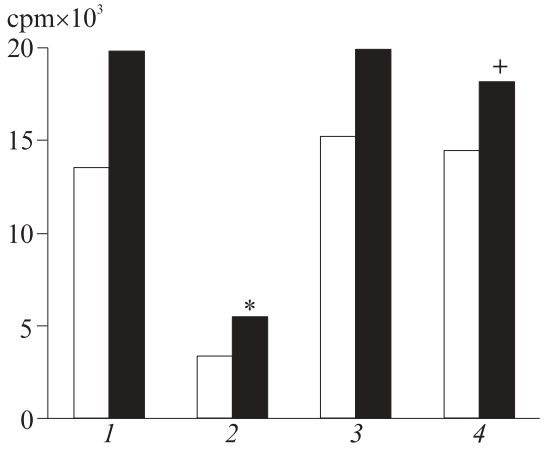

Fig. 3. Blast transformation of lymphocytes from rats exposed to cold stress and receiving prolactin: intact animals (*1*), stressed animals (*2*), stressed animals receiving prolactin in a dose of 40 ng per 100 g body weight (*3*), and stressed animals receiving prolactin in a dose of 40 ng per 100 g body weight 20 min before stress (*4*). Light bars: incubation with Con A (0.625 μg/ml). Dark bars: incubation with Con A (0.625 μg/ml) and native IL-1 (0.06 μg/ml). Ordinate: incorporation of ^{3}H-thymidine into cell DNA over 1 min. $p<0.05$: *compared to intact animals; +compared to stressed animals.

response. Pretreatment with prolactin in a dose of 25 ng per 100 g body weight 20 min before stress prevented suppression of the immune response. This effect was not observed after administration of prolactin in a dose of 5 ng per 100 g body weight (Fig. 1).

These data indicate that prolactin in the specified doses produces an immunoprotective effect during stress. We studied the effect of prolactin on peritoneal macrophages (first elements of protection) and production of LAF during cold stress. Immunomodulating cytokines IL-1, IL-6, and tumor necrosis factor-α are the components of LAF produced by macrophages. A major component is IL-1. It should be emphasized that supernatants of resident macrophages have no LAF activity [7].

Administration of prolactin to nonstressed rats was followed by spontaneous production of LAF over the first day postinjection. Additional stimulation of macrophages with LPS *in vitro* increased the intensity of LAF production by 1.5-2.0 times (Fig. 2).

Spontaneous production of LAF is induced by stress [1]. Pretreatment with prolactin initiated the release of LAF 10 min and 24 h after stress. Under these conditions the intensity of LAF secretion 1.5-2.0-fold surpassed that observed after stress. Therefore, prolactin significantly activated macrophages and intensified production of LAF. Prolactin abolishes suppression of LAF secretion after application of exogenous glucocorticoid hormones [2], which correlates with activation of immunological reactions and immune response.

Prolactin stimulates the release of immunomodulating and lymphocyte-activating cytokines (*e.g.*, IL-1) from macrophages on stressed animals. These data expand our knowledge of the involvement of immunomodulating hormones and cytokines in the protective response. The effectiveness of protective reactions depends not only on the amount of IL-1 and other cytokines, but also on the sensitivity of the target cells to these compounds. This characteristic determines the intensity of cell proliferation [1]. Peripheral blood lymphocytes undergo blast transformation in response to treatment with IL-β in the presence of lectins in the suboptimal dose [7]. These changes reflect the comitogenic effect of IL-β characterizing proliferative activity of lymphocytes.

Intraperitoneal injection of prolactin in doses of 5, 25, and 40 ng per 100 g body weight had no effect on blast transformation response to native IL-β (compared to intact animals, Fig. 3). Peripheral blood lymphocytes from rats taken 10 min after cold stress did not undergo blast transformation in response to native IL-1β. It was manifested in decreased incorporation of labeled thymidine incorporation into lymphocyte DNA (Fig. 3).

Severe stress causes immunosuppression, which is accompanied by an increase in blood glucocorticoid hormone concentration, decrease in lymphocyte sensitivity to IL-1, and inhibition of lymphocyte proliferation. These changes were not observed 24 h after stress [2]. Administration of prolactin in a dose of 40 ng per 100 g body weight 20 min before stress prevented the decrease in proliferative activity of lymphocytes isolated from animals 10 and 24 h after stress. These data show that prolactin has no effect on proliferation of cells from intact animals in the reaction of blast transformation. During stress prolactin increases the sensitivity of lymphocytes to the comitogenic effect of IL-1 and prevents the stress-induced decrease in proliferative activity of rat peripheral blood lymphocytes. It can be hypothesized that prolactin counteracts the destabilizing effect of glucocorticoids on lymphocyte proliferation during stress, produces a permissive effect, and increases regulatory activity of IL-1. These properties probably determine the immunomodulating effect of prolactin during stress [3,7]. The effects of prolactin, glucocorticoid hormones, and IL-1 are probably integrated during the ligand-receptor interaction, which prevents the development of immune dysfunction during stress.

Thus, systemic administration of prolactin increases the sensitivity of peripheral blood lymphocytes to the regulatory effect of IL-1 during stress and intensifies proliferation of lymphocytes. Activation of mononuclear phagocytes and intensive production of immunomodulating cytokines in the composition of LAF (the major component IL-1) correlate with variations in humoral immune reactions. These changes can be considered as a mechanism for the immunoprotective effect of prolactin during stress.

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