

PROGESTERONE INHIBITS MAST CELL SECRETION

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Mast cells are involved in allergic reactions, where they secrete numerous vasoactive, inflammatory and nociceptive mediators in response to immunoglobulin E (IgE) and antigen. However, they have also been implicated in inflammatory conditions, such as painful bladder syndrome/interstitial cystitis (PBS/IC), irritable bowel syndrome (IBS) and migraines, all of which occur more often in women and are exacerbated during ovulation, but are suppressed during pregnancy. Mast cells express high affinity estrogen receptors and estradiol augments their secretion, while tamoxifen inhibits it. Here we report that progesterone (100 nM), but not the structurally related cholesterol, inhibits histamine secretion from purified rat peritoneal mast cells stimulated immunologically or by substance P (SP), an effect also documented by electron microscopy. These results suggest that mast cell secretion may be regulated by progesterone and may explain the reduced symptoms of certain inflammatory conditions during pregnancy.

Mast cells derive from the bone marrow and differentiate under local tissue microenvironmental conditions (1). They are necessary for the development of allergic reactions, during which they secrete numerous vasoactive and pro-inflammatory mediators (2). In addition, mast cells are also involved in neuroinflammatory conditions (3). Many such conditions are exacerbated by estrogens (4-7) and/or are associated with a state of progesterone deficiency (8).

A relevant case is painful bladder syndrome/interstitial cystitis (PBS/IC), a sterile bladder condition of unknown etiology, which occurs almost exclusively in women and is characterized by diffuse pelvic pain, as well as urination frequency, urgency and nocturia (9). These symptoms worsen

during the menstrual cycle in most pre-menopausal women, but subside in late pregnancy (10).

There is now clear evidence of increased and activated bladder mast cells in PBS/IC (11), as well as proliferation of sensory nerves (12), especially those positive for substance P (SP) (13). Moreover, SP and carbachol (14) can trigger bladder mast cell secretion, processes augmented by estradiol (14-15). Mast cell mediators could damage the bladder wall and activate local nerve endings, thus causing irritative voiding symptoms and pain.

Human leukemic (HMC-1) cells and bladder mast cells were shown to express high affinity estrogen receptors, which were increased in PBS/IC (16); however, these same bladder mast cells were almost devoid of progesterone receptors (17). An

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imbalance of estrogen and progesterone receptors, together with corresponding high circulating estrogen and low progesterone serum levels, may possibly explain the high incidence of PBS/IC in women and its worsening perimenstrually. Moreover, such an imbalance may also explain why symptoms decrease during late pregnancy, when progesterone levels are particularly high (18). Here we report that progesterone inhibits mast cell secretion.

MATERIALS AND METHODS

Purified rat IgE and mouse anti-rat IgE were obtained from Zymed Laboratories Inc. (South San Francisco, CA). Compound 48/80 (C48/80), substances P, cholesterol, progesterone, phosphatidyl serine and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO).

Isolation and purification of mast cells

Rat peritoneal mast cells were obtained from male Sprague-Dawley rats, Taconic Inc. (Germantown, NY), weighing approximately 350g. Mast cells were purified (>90% purity) in Hepes-buffered Locke's solution, pH 7.2 (150 mM aCl, 5 mM KCl, 5 mM Hepes, 2 mM CaCl₂, 1 g dextrose and 1 g bovine serum albumin per liter, pH 7.2), over 22.5% Metrizamide (Accurate Scientific, NY) (19), washed twice and re-suspended in the same buffer. For histamine release, 500 µl of buffer was added to the pellets after removal of the supernatant fluid; both pellets and supernatant fluid were acidified with perchloric acid (final concentration 2%), boiled and centrifuged to remove the precipitated protein, and histamine was measured with a fluorometric assay. Net release was calculated by dividing the amount of mediator released during stimulation, minus that present in unstimulated controls, by that in the supernatant and pellet combined. Percent secretion or percent inhibition was calculated accordingly.

Immunologic stimulation

For immunologic stimulation, 10 µl of purified rat IgE and 100 µg/ml sodium phosphatidyl serine were added to the cells (100 µl containing 100,000 cells). After two washes, the cells were incubated with or without progesterone or cholesterol at the indicated concentrations for 90 min at 37°C, following which mouse anti-rat IgE (7% final concentration) was added and the cells were incubated for an additional 30 min. The reaction was terminated by the addition of cold Locke's buffer and centrifugation.

C48/80 stimulation

Aliquots of mast cells (100 µl) were incubated with

or without progesterone or cholesterol at the indicated concentrations for 90 min at 37°C. The cells were then incubated with C48/80 (0.3 µg/ml) for an additional 15 min. The reaction was terminated by addition of cold Locke's buffer and centrifugation.

SP stimulation

This procedure was similar to that used for C48/80, except that the incubations with the SP (5×10^{-5} M) were done in calcium-free Locke's buffer containing 0.1 mM EDTA.

Cell viability

Mast cells (10^6 cells/ml) were combined (9:1) with 20 mg/100 ml Trypan blue for 5 min at 24 °C, washed once and observed at 400× with a Nikon Diaphot inverted light microscope.

Pre-treatment with cholesterol or progesterone

Mast cells were pre-incubated with cholesterol or progesterone at the indicated concentrations for 90 min at 37°C before stimulation. Unlike progesterone, which was soluble in ethanol, cholesterol was dissolved in DMSO because it was not readily soluble in ethanol. The final solvent concentration (0.1%) had no effect on mast cell secretion or viability.

Electron microscopy

Mast cells were fixed, stained, processed and examined as previously described (20).

Statistics

Results are presented as the mean $\pm$ standard deviation and are compared using the two-tail Student's *t* test. Significance is denoted by $p < 0.05$.

RESULTS

Basal mast cell histamine release was $1.9 \pm 0.3\%$ in male and $3.0 \pm 0.4\%$ in female rats, respectively. Treatment of mast cells for 90 min with progesterone alone up to 100 nM did not significantly affect the release of histamine (2.6 ± 0.3), whether male ($n=10$) or female ($n=4$) rats were used (results not shown).

Pre-treatment of mast cells for 90 min with 100 nM cholesterol alone, had no effect on basal histamine release or that induced by 0.3 µg/ml C48/80 (Fig. 1). However, similar pre-treatment with progesterone reduced histamine release induced by C48/80 from $54.9 \pm 9.7\%$ to $27.5 \pm 9.0\%$ ($p < 0.05$). The effect of progesterone was also tested against

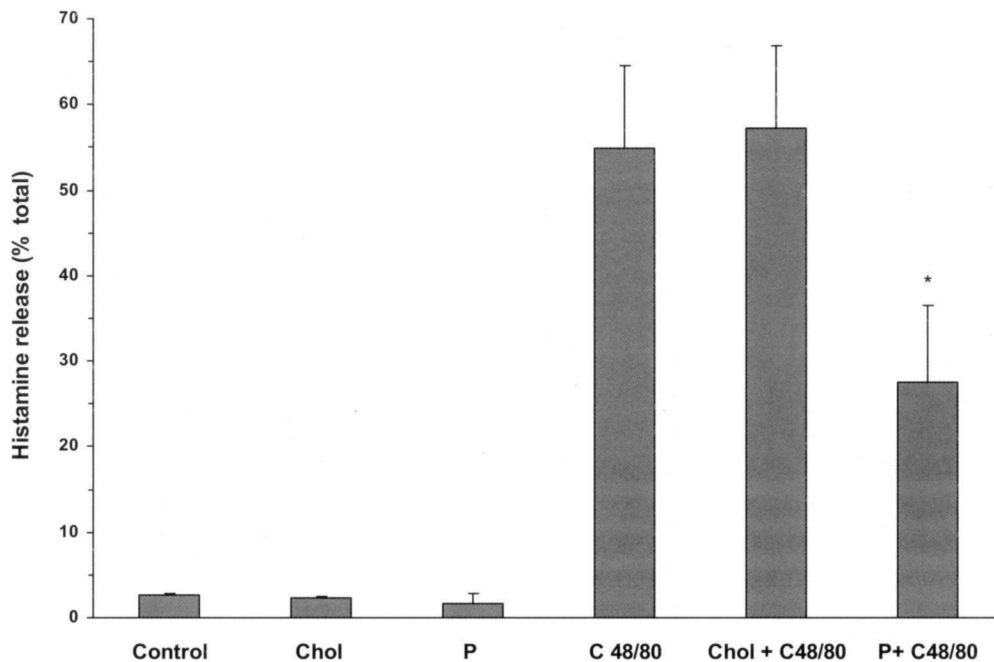


Fig. 1. Effect of progesterone (P) and cholesterol (chol) at 100 nM on C48/80-induced (0.3 µg/ml) histamine release. (* $p < 0.05$).

mast cell stimulation by IgE and antigen, as well as by SP (Fig. 2). Progesterone at 100 nM inhibited immunologic stimulation of histamine by 55%, C48/80-induced release by 52% and SP-induced release by 78% (Fig. 2). Only immunologic stimulation of histamine release was inhibited by 14% and 19% at 10 nM and 1nM respectively, (Fig. 2).

On transmission electron microscopy, control mast cells showed numerous, homogeneous, intact, electron dense secretory granules (Fig. 3A). Cells treated with 100 nM progesterone alone for 90 min had largely unaffected secretory granules and looked similar to control cells (Fig. 3B). Cells stimulated with 0.3 µg/ml C48/80 showed large exocytotic cavities and many swollen granules which had totally lost their electron density (Fig. 3C). Cells treated with 100 nM progesterone for 90 min before stimulation with C48/80 had >60% of their granules intact, as compared to less than 20% in those stimulated with C48/80 (Fig. 3D and E).

DISCUSSION

The biochemical and ultrastructural findings presented indicate that progesterone can inhibit

rat mast cell secretion. The inhibitory effect of progesterone appears to be unique as it is not apparent with other structurally similar compounds, such as cholesterol. Testosterone also inhibited secretion at similar concentrations, while β -17 estradiol augmented mast cell secretion (15). Corticosteroids have no acute effect, but require at least 24 hr incubation in culture for any inhibitory effect to become evident (21). The lowest progesterone concentration capable of some inhibition (1 nM) is still higher than the mean serum progesterone found in the third trimester of pregnancy (150 µg/L) (18) and the mean post-ovulatory levels (25 µg/L) (22). Human mast cell expression of progesterone receptors may vary with the ovulatory or disease state. The relative number of estrogen and progesterone receptors on mast cells may then regulate the extent of mast cell secretion.

The present results may explain why autoimmune diseases usually subside during late pregnancy (4-7). Previous studies support our findings. Progesterone inhibited 48/80-induced histamine-dependent hyperalgesia in rodents (23). Some progesterone-like chemicals from sponges were also shown to inhibit mast cell secretion (24). Progesterone was

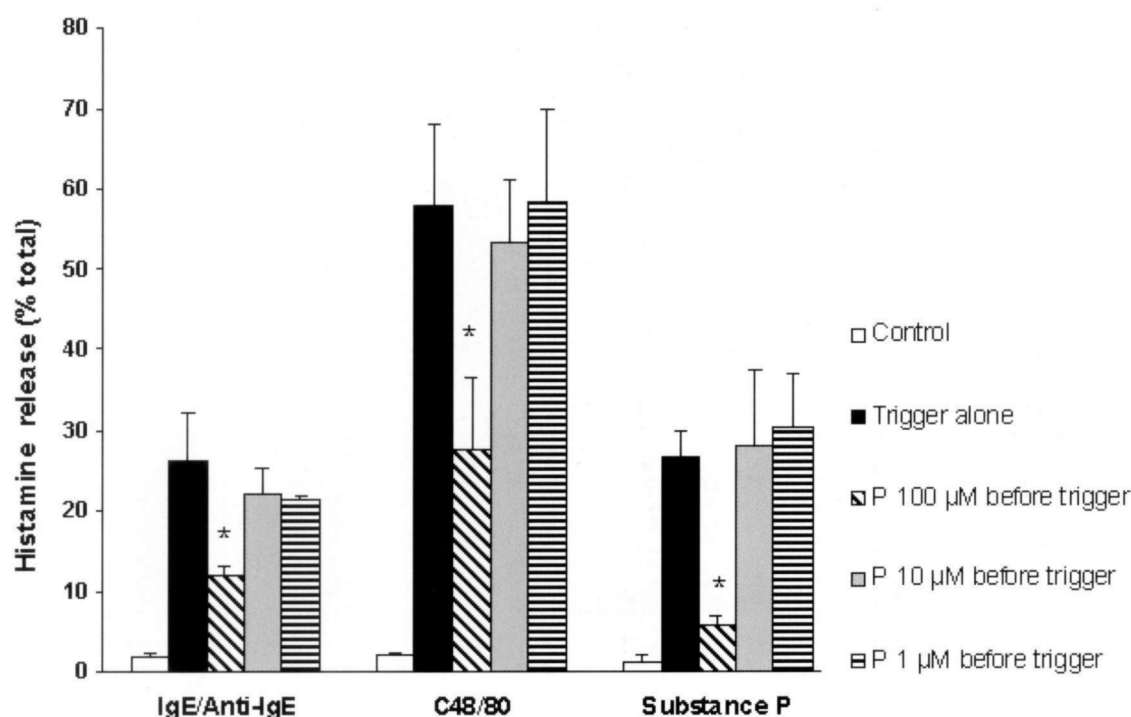


Fig. 2. Dose-response of progesterone on histamine release induced by (a) anti-IgE, (b) C48/80 (0.3 µg/ml) and (c) SP (100 nM). (* $p < 0.05$).

also shown to significantly inhibit lymphocyte activation in the luteal phase (25 µg/L serum progesterone) but not in the proliferation phase (0.15 µg/L serum progesterone) (25). Moreover, the number of intact uterine mast cells and the histamine content were increased during pregnancy but were sharply reduced post partum (26), implying that mast cell activation was inhibited during pregnancy. High affinity binding of progesterone has been reported on rat basophil leukemia (RBL-1) cells (27), which are equivalent to mucosal mast cells (28).

Asthma (29-30) and allergies (31) appear to be exacerbated by female sex hormones. Asthma is better controlled during pregnancy, presumably due to high progesterone levels. Uterine histamine secretion apparently occurs prior to ovulation (32), which may help explain why symptoms of PBS/IC and other autoimmune disorders worsen during the menstrual cycle (10). Estradiol can release histamine *in vivo* (33-34) and *in vitro* (15). Moreover, mast cells proliferate in the presence of estradiol in the uterus and the guinea pig bladder (35), but they are reduced in numbers during pregnancy (36). Estradiol

enhances the functional activity of rat mast cells, while progesterone substantially inhibits this effect (37). A state of "progesterone deficiency" may occur in rheumatoid arthritis (8), but progesterone inhibits the differentiation of cytotoxic effector cells (38) and results in the suppression of allograft rejection (39). PBS/IC is characterized by increased and activated bladder mast cells (11), but while mast cells express estrogen receptors (16), less than 30% of all bladder mast cells in PBS/IC are positive for progesterone receptors (17).

Mast cells participate in immediate and late phase reactions, during which they release numerous mediators, either upon exposure to IgE and specific antigen or in response to a variety of neuropeptides and other triggers (2-3). Histamine, bradykinin, prostaglandin D₂, cytokines and tryptase could cause vasodilation, smooth muscle contraction, inflammation, tissue damage, neural stimulation and pain, thus explaining many of the symptoms and the endoscopic appearance of patients with PBS/IC (9). Of these patients, two thirds have allergy problems, while almost 40% appear to also suffer from IBS (40),

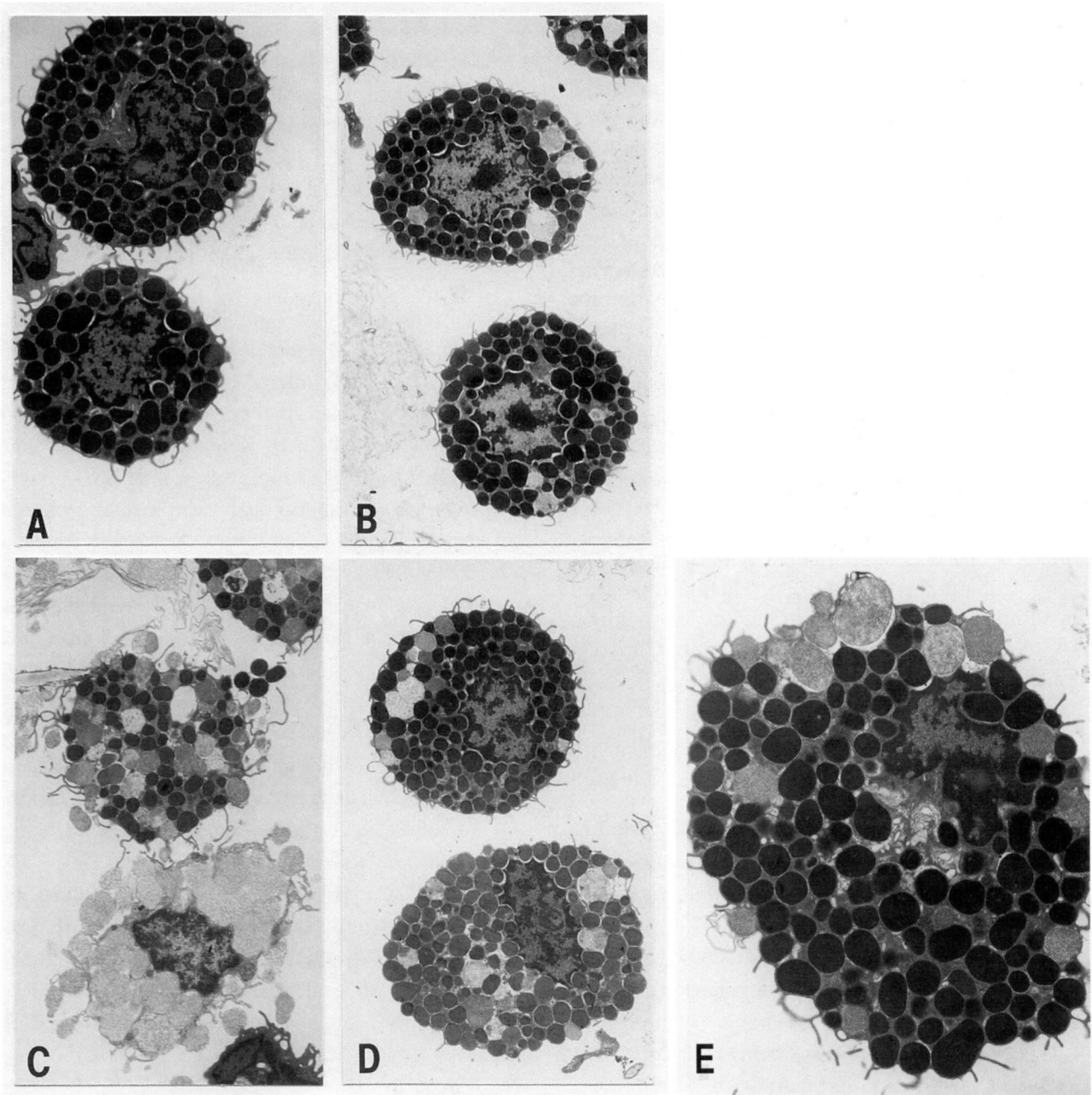


Fig. 3. Transmission electron micrographs of purified mast cells incubated with progesterone. (A) Control mast cells. Note the numerous homogeneous, electron dense secretory granules. Magnification = $\times 4,500$. (B) Mast cells treated with 100 nM progesterone for 90 min at 37° C. Note that almost all the secretory granules are intact. Magnification = $\times 4,500$. (C) Mast cells treated with 0.3 $\mu\text{g/ml}$ C48/80 for 15 min at 37° C. Note massive degranulation with large exocytotic cavities containing swollen granules, which have lost their electron density. Magnification = $\times 4,500$. (D) Mast cells pretreated with 100 nM progesterone for 90 min at 37° C, before treatment with 0.3 $\mu\text{g/ml}$ C48/80 for another 15 min. Note the lack of any exocytotic cavities and the presence of numerous intact granules even though some of them have lost their electron density. Magnification = $\times 4,500$. (E) High magnification of mast cells treated as in D above to appreciate that most of the secretory granules are intact. Magnification = $\times 20,400$.

conditions which occur more frequently in women. Mast cells are located close to neuronal processes (17, 41-42), often positive for SP (43), which are increased in PBS/IC (43). In fact, SP can stimulate mast cell secretion directly or through nerve activation (44). Moreover, the stimulating effect of estradiol on mast cell function was found to be partially dependent on an intact nervous system (37).

The present results may help to explain the high prevalence of certain neuroimmunoendocrine disorders in women, the worsening of symptoms perimenstrually, as well as the decrease of symptoms during pregnancy. High progesterone containing oral contraceptives may be a useful adjuvant therapy, together with other immunomodulators (45).

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