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Selective modulation of lymphoproliferation and cytokine production via intracellular signaling targets by α_1- and α_2-adrenoceptors and estrogen in splenocytes

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ABSTRACT

The mechanistic implications of the presence of sympathetic noradrenergic innervation in lymphoid organs in synaptic association with lymphocytes open to the influence of hormonal fluctuations throughout reproductive age in females has not been investigated yet.

Objectives: The aim of the present study is to investigate the role of alpha-adrenoceptors (α-ARs) and estrogen in modulating immune responses in the spleen through intracellular signaling targets such as ERK 1/2, CREB, Akt, NF-κB.

Methods: Splenocytes from young Sprague-Dawley rats were incubated with α_1- and α_2- AR specific agonists, phenylephrine and clonidine, without and with 17b-estradiol or specific antagonists prazosin and idazoxan to examine their effects on proliferation, cytokine production, nitric oxide production, and intracellular signaling molecules.

Results: α_1-AR stimulation inhibited lymphocyte proliferation and IFN-g production and enhanced IL-2, p-ERK and p-CREB expression. Co-stimulation using estrogen enhanced cytokine production and suppressed p-Akt expression. α_1-AR blockade reversed agonist-induced IL-2 production alone. α_2-AR stimulation inhibited lymphocyte proliferation, p-ERK and p-CREB expression, and increased p-NF-kB and p-Akt expression. Co-stimulation with estrogen increased IL-2 and suppressed p-CREB expression. α_2-AR Idazoxan prevented IL-2 production in the absence and presence of estrogen, and reversed clonidine-induced increase in NO production and p-ERK and p-Akt expression in the presence of estrogen.

Conclusions: These results suggest that the cell-mediated immune responses are selectively modulated depending upon the subtypes of α-AR and further, these effects are differentially regulated in the presence of estrogen mediated through selective alteration in the intracellular signaling pathways involving ERK, CREB, Akt, and NF-κB.

1. Introduction

The central nervous system regulates immune reactivity through neuroendocrine outflow via hormones, sympathetic noradrenergic innervation of lymphoid organs and also, through immune molecules from the lymphoid organs that cross the blood–brain barrier to influence the central nervous system functions [1,2]. Several reports have established the presence of sympathetic noradrenergic (NA) nerves in primary and secondary lymphoid organs of male mice and rats and female rats, and that these nerves release norepinephrine (NE) to influence immune responses [1–5]. The distributions of α- and β-adrenergic receptors (ARs) on the immune cells are lymphoid organ-specific and have different affinities for the released NE that mediate the neural signals of NE to alter the immune responses [2–4]. The expression of β2-AR is predominantly found on the lymphoid cells of rodents and humans while α_1- and α_2-ARs are have been reported either to be induced by cytokines and hormones or normally expressed on natural killer (NK) cells and T and B lymphocytes [4,6–8]. Binding of NE to α_1- and α_2-ARs on lymphocytes influences lymphocyte proliferation, T helper (Th1 and Th2) cytokine production, apoptosis of lymphocytes and increases IgM antibody production [8–11]. However, the role of α_1- and α_2-ARs in altering immune reactivity especially, in the presence of estrogen is not known although centrally they are known to modulate the female reproductive behavior, lordosis [12].

Female rats attain puberty around 35–40 days of age, exhibiting a regular 4-day estrous cycle followed by irregular estrous cycles by 8–10 months of age, constant estrus stage (10- to 19-month) marked

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by high circulating estrogen (E), persistent diestrus stage with increased circulating progesterone (P) and then, anestrus stage by19- to 30-month of age [13,14]. These progressive alterations in estrous cycle patterns with increasing age are associated with the development and growth of mammary tumors and autoimmune diseases [15,16]. Fluctuations in circulating gonadal hormones during the various stages of estrous cycle influence T and B cell proliferation, and localization of IgA-producing plasma cells [17]. Estrogen is known to play a key role in inflammatory process by altering Th1/Th2 cytokine balance and thereby, influencing the development of various female-specific diseases such as hormone-dependent cancer, autoimmune diseases, and osteoporosis [18]. Estrogen-induced effects on proinflammatory cytokines such as TNF-α by peritoneal macrophages may be mediated through α_2-AR [19] in peripheral nervous system to affect cell-mediated immune responses.

Therefore, in the present study, we have investigated the in vitro effects of different concentrations of α_1- and α_2-AR agonists, phenylephrine and clonidine, respectively on proliferation of splenic lymphocytes and cytokine (IL-2 and IFN-γ) production in the absence and presence of 17β-estradiol to establish their effects on cell-mediated immune responses. To determine the specificity of their actions on cell-mediated immune responses, specific α_1- and α_2-AR antagonists, prazosin and idazoxan, respectively were coincubated in the absence and presence of α_1- and α_2-AR agonists. In addition, we examined the levels of molecular signaling factors such as p-ERK 1/2, p-CREB, p-Akt, and p-NF-κB in the splenocytes to understand the molecular actions of α_1- and α_2-AR agonists in the absence and presence of estrogen. Simultaneously, the NO production was measured to determine the role of this compensatory factor on α_1- and α_2-AR and estrogen-induced modulation of immune reactivity. We report here that α_1- and α_2-AR agonists suppress lymphocyte proliferation and differentially regulate cytokine production and phosphorylation of ERK, CREB, Akt, and NF-κB in the absence and presence of estrogen.

2. Materials and methods

2.1. Animals

Young (3-month-old; n = 18) male Sprague–Dawley rats (National Institute of Nutrition, Hyderabad, India) were purchased and housed in the animal house at SRM University for a period of one week. Following acclimatization, the animals were decapitated at 08:00 h and the spleens were aseptically dissected and placed in sterile tubes containing HBSS for isolation of lymphocytes for in vitro experiments with adrenergic agonists and antagonists in the presence and absence of estrogen. The experiments were done thrice using 6 rats/trial. The experiments were conducted in accordance with the principles and procedures outlined and approved by the Institutional Animal Ethics Committee.

2.2. Isolation of lymphocytes

Lymphocytes were prepared as described previously [20,21]. Briefly, a block of spleen was aseptically transferred to a stomacher bag with HBSS (Sigma) and homogenized using a stomacher. The cell suspension thus obtained was passed through a nylon mesh to remove large aggregates followed by repeated washes with HBSS. Cells obtained after the wash were then carefully layered on Histopaque 1077 (Sigma-Aldrich, St. Louis, MO), after removing the lymphocytes from the Histopaque/HBSS interface and washed thrice with HBSS, the cells were then re-suspended in RPMI 1640 medium supplemented with 5% fetal calf serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 0.01 mM nonessential amino acids, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, 100 mg/ml streptomycin, 24 mM sodium bicarbonate, and 10 mM HEPES for in vitro culture.

2.3. Treatment

2.3.1. Experiment 1

Splenic lymphocytes (2×10^5 cells/ml) were treated with different doses (10^{-3} M, 10^{-6} M and 10^{-9} M) of α_1-AR specific agonist, phenylephrine, in the presence and absence of α_1-AR specific antagonist, prazosin (10 μM), and different doses (10^{-3} M to 10^{-9} M) of α_2-AR specific agonist, clonidine, in the presence and absence of α_2-AR specific antagonist, idazoxan (10 μM), in 24-well and 96-well culture plates in the presence and absence of 10^{-9} M 17β-estradiol. The doses of α-AR antagonists and 17β-estradiol were selected from a preliminary study in splenic lymphocytes. The plates were kept in a humidified chamber with 5% CO_2 at 37 °C to measure the effects of α-AR and estrogen on cytokines and T lymphocyte proliferation. Adrenergic agonists and antagonists were freshly prepared every day as 0.1 M stock solutions in 10 mM L-ascorbate and serially diluted to the aforementioned concentrations using media. Alpha-AR agonists and antagonists were purchased from Sigma-Aldrich, St. Louis, MO.

2.3.2. Experiment 2

Adrenergic receptor-specific effects on downstream signaling molecules was assessed by co-incubating different doses (10^{-3} M to 10^{-9} M) of α_1- and α_2-AR specific agonists with or without their specific antagonists (10^{-5} M) in 24-well and 96-well culture

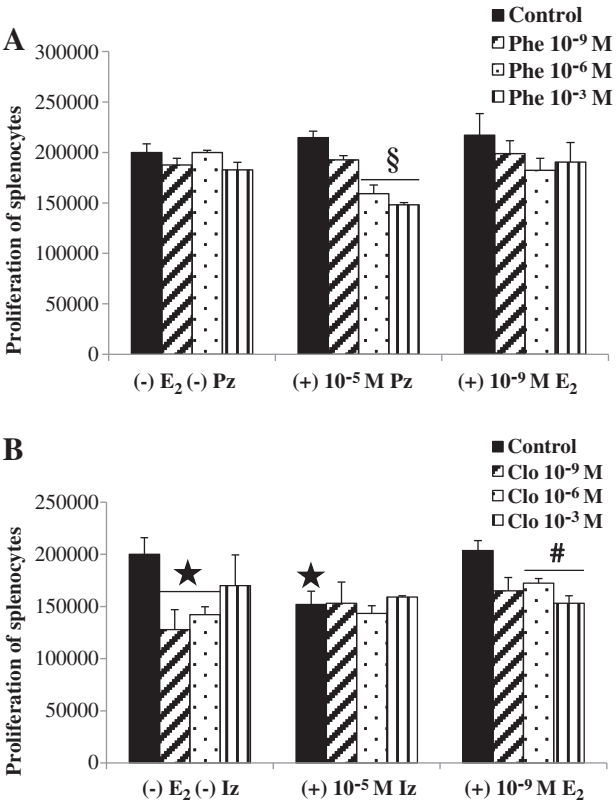

Fig. 1. In vitro addition of α_1- and α_2-AR agonists and antagonists in the absence and presence of 10^{-9} M 17β-estradiol on T cell proliferation in the spleen. Co-incubation with α_1-AR agonist, phenylephrine did not alter the proliferation of splenocytes, although co-treatment with α_1-AR-specific antagonist prazosin significantly decreased it (A). Treatment of splenic lymphocytes with α_2-AR agonist, clonidine, decreased proliferation irrespective of antagonist, idazoxan (Iz), co-treatment but co-incubation with 17β-estradiol reversed the agonist-mediated (Clo 10^{-6} M) decline (B). *$p < 0.05$ compared to control. §$p < 0.01$ compared to respective agonist-treated group. #$p < 0.05$ compared to 10^{-9} M 17β-estradiol-treated group.