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# RELATIONSHIP OF INTERLEUKIN-2 AND PROLACTIN IN RAT'S SERUM IN RESPONSE TO CHANGES IN PROLACTIN LEVELS

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## SUMMARY

In order to induce changes in serum prolactin levels, estradiol benzoate as a hyperprolactinemic agent, and bromocriptine as an antiprolactin drug were used in this stduy. 80 immature male and female rats were divided randomly into five groups for each sex (8 animals for each group). The first group of both male and female rats were considered as control which were daily injected s/c with sesame oil for 10 days. The rest groups were daily injected s/c with estradiol benzoate for 10 days. After 24 hrs. from the last injection, the animals of the 2nd group of both male and female rats were sacrificed in the morning and the obtained sera stored at -20°C till analysis. The 3rd, 4th and 5th groups were followed by injection of bromocriptine at three dose levels (10, 20 and 40 µg/animal) for 7 days. After 24 hrs from the last injection, the animals were slaughtered and the obtained sera were kept at -20° till analysis. The changes in serum interlaukin-2 levels were determined using ELISA technique and the levels of prolactin were measured using RIA kit. The serum levels of both interleukin-2 and prolactin revealed a significant increase of (308% and 62%) and (146% and 89%) respectively in male and female immature rats injected with estradiol benzoate (2nd group). In animal groups which were followed by injection of bromocriptine at doses of (10, 20 and 40 µg / animal), the serum levels of interleukin-2 and prolactin showed a significant decrease of (10% and 15%), (41% and 49%) and (84% and 75%) orderly in male rats, and (21% and 25%). (56% and 63%) and (86% and 89%) respectively in female rats. Furthermore, the results of the present study revealed a high positive correlation coefficient between interleukin-2 and prolactin.

## INTRODUCTION

A large body of data indicates that the pituitary hormone prolactin is a regulator of immune function in keeping with its structural analogy to the other member of the cytokine superfamily (Matera et al., 1996). Impaired lymphocyte response has been shown to accompany both congenital and experimentally induced derangement of prolactin release and administration of prolactin reverses immune depression (Matera, 1996). Moreover, interleukin-2 plays the crucial role in the immune system operation. It is a lymphokine, produced mainly by T-lymphocytes activated from a resting

state by interleukin-1 and interleukin-6 released from macrophages (Mizel, 1989). In vitro studies have shown that prolactin can replace or interact with interleukin-2 during T-lymphocyte activation (Matera, 1996). Both prolactin and interleukin-2 are potent mitogens for Nb2 rat T-lymphoma cell line (Croze et al., 1988), and the interaction of these factors with the cognate ligands on Nb2 cells activates the transcription factors that recognize similar DNA target sites (Gilmour and Reich, 1994). Other experimental evidence points to a sequential action of interleukin-2 and prolactin during T-cell proliferation. It has been shown that antiprolactin antisera inhibt the proliferation response of murine and human T and B cells (Hartmann et al., 1989), and of human peripheral blood mononuclear cells to mitogens (Sabarwal et al., 1992). Moreover, the findings of Montgomery et al. (1992) demonstrated that human thymocytes and peripheral T-cells (but not B cells or monocytes) are able to synthesize and secrete their own prolactin. These findings and the results of Moraes et al. (1995) led to postulate that not only pituitary but also autocrine or paracrine prolactin production by the lymphocytes themselves and/or cytokines or antigen can play the major role in the regulation of prolactin receptor expression on cells of the immune system. This hypothesis is supported by: (1) the demonstration that T-cells mitogens can modulate prolactin-receptors expression in human peripheral blood lymphocytes (Dardenne et al., 1994), (2) data of Moraes et al., (1995) showing no correlation between prolactin serum levels and prolactin receptor expression, and (3) also the data of Moraes et al. (1995) revealed that the frequency of prolactin receptor cells is not significantly modified by the decrease in circulating prolactin level.

The immunoregulatory effects of prolactin appear to be complex as it stimulates or suppresses various immune functions depending upon the species, parameters studied and concentration (Gala, 1991). In hypohysectomized animals and in animals treated with bromocriptine contact sensitivity responses, T-cell proliferative responses and secretion of interferon-γ were reported to be inhibited (Bernton et al., 1988). Furthermore, it was found that prolactin induces interleukin-2 receptor expression in murine lymphocytes (Gala and Shevach, 1993) and is necessary for interleukin-2 induced lymphocyte proliferation (Clevenger et al., 1990). On the other hand, in T-cell lines, interleukin-2 has been reported to stimulate a seven-fold increase in prolactin receptors and two-fold increase in the mRNA for prolactin receptor (Clevenger et al., 1991). The main aim of the present investigation was designed to examine the changes in the levels of interleukin-2 in response to induced changes in prolactin levels.

## MATERIAL AND METHODS

80 immature male and female albino rats weighing 160-180 g (40 animals for each sex), kept under natural photoperiod of 14 hrs light/10 hrs dark with temperature range 28-32°C and supplied with tap water and food available ad libitum, were used in this investigation. The animals were divided into five groups for each sex, 8 animals for each group. The first group of both sexes was considered as control. They were

injected s/c with 0.2 ml sesame oil for 10 days. The other groups were daily injected s/c with 1.7 µg estradiol benzoate dissolved in 0.2 ml sesame oil/animal for 10 days to induce hyperprolactinaemia (Heiman and Ben Jonathan, 1982). The second group of both male and female rats was sacrificed in the morning after 24 hrs from the last injection and the obtained sera were kept at -20°C till analysis. In the last three groups, estradiol benzoate injection was substituted by daily s/c injection with bromocriptine mesilate (Sandoz) at doses of 10 µg (3rd group), 20 µg (4th group) and 40 µg/animal (5th group) for 7 days. Each dose was dissolved in 0.5 mly ethanol/0.9% Na Cl (3:2) according to Ravault et al., (1982). After 24 hrs from the last injection, all animal groups were sacrificed in the morning and the obtained sera were kept at -20°C till analysis. The serum levels of interleukin -2 were measured using the Predicta Interleukin-2 ELISA kit according to Grimm (1983), and the sera levels of prolactin were determined by using RIA technique according to Jacobs et al. (1990). The data were statistically analysed using ANOVA and correlation coefficient (r) according to Snedecor and Cochran (1979).

## RESULTS AND DISCUSSION

Interleukin-2 is a pluripotential cytokine that besides its role in the regulation of immunocompetent cells function, it also stimulates hormone secretion. On the other hand, several factors including cytokines (interleukin-1 and interleukin-6) and pituitary hormones including thyrotropin and prolactin exert stimulatory effects on the T-cell connected interleukin-2 production (Komorowski et al., 1994). In the present study, both serum interleukin-2 and prolactin levels showed a significant increase of (308% and 62%) and (146% and 89%) respectively in male and female rats (2nd group). While in animal groups injected with 10, 20 and 40 µg of bromocriptine, the serum interleukin-2 and prolactin levels revealed a significant decrease of (10% and 15%), (41% and 49%) and (84% and 75%) orderly in male rats and (21% and 25%), (56% and 63%) and (86% and 89%) respectively in female rats (Tables 1 & 2 and Figures 1 & 2). These results are consistent with several studies which suggest that bromocriptine has immunomodulatory properties and the bromocriptine therapy or hypophysectomy can suppress inflammation in the adjuvant arthritis model and that this effect can be overcome with the concurrent administration of prolactin (Berczi et al., 1984). Furthermore, the in vivo administration of bromocriptine has been documented to inhibit T-cell proliferation and production of cytokines (Bernton et al., 1988). In addition, antiprolactin antiserum has been demonstrated in vitro to inhibit the concanavalin A and lipopolysaccharide-driven proliferation of murine and human lymphocytes (Hartmann et al., 1989). The presence of prolactin receptors on lymphocytes (Montgomery et al., 1987), the secretion of prolactin-like molecule by concanavalin-A-stimulated lymphocytes which is critical for progression through cell cycle (Hartmann et al., 1989), and the upregulation of interleukin-2 receptors on T-cells induced by prolactin stimulation (Mukherjee et al., 1990) further suggests an immunoregulatory role for

Table (1): Serum interleukin-2 and prolactin levels, the change % of both factors, (r) between the two parameters after each treatment in immature male rats injected daily with estradiol benzoate ($E_2$) for 10 days (2nd group) followed by injection of bromocriptine for 7 days at three different doses (3rd, 4th and 5th groups).

| | Control 1st group | $E_2$ 2nd group | Bromocriptine 3rd group | Bromocriptine 4th group | Bromocriptine 5th group |
|---|---|---|---|---|---|
| Interleukin-2 pg/dl | 130.2±7.6$^a$ | 530.8±32$^b$ | 478.4±21.6$^c$ | 313.4±14.7$^d$ | 82.5±5.3$^e$ |
| Change % | | +308* | -10** | -41** | -84** |
| Prolactin ng/dl | 52.4±3.8$^a$ | 84.6±6.2$^b$ | 71.7±5.3$^c$ | 43.5±3.2$^a$ | 20.8±3.6$^d$ |
| Change % | | +62* | -15** | -49** | -75** |
| r | + 0.93 | +0.90 | +0.94 | +0.96 | +0.91 |

Mean ±SE

a,b,c,d and e = Means in the same rows sharing the same subscript are not significant at $p \leq 0.05$

* = Change % in comparison to the control (1st group).

** = Change % in comparison to the $E_2$ (2nd group).

\+ = Increase in the change % or positive (r). - = Decrease in the change %

Table (2): Serum interleukin-2 and prolactin levels, the change % of both factors, (r) between the two parameters after each treatment in immature female rats injected daily with estradiol benzoate ($E_2$) for 10 days (2nd group) followed by injection of bromocriptine for 7 days at three different doses (3rd, 4th and 5th groups).

| | Control 1st group | $E_2$ 2nd group | Bromocriptine 3rd group | Bromocriptine 4th group | Bromocriptine 5th group |
|---|---|---|---|---|---|
| Interleukin-2 pg/dl | 214.4±13.3$^a$ | 527.8±21.7$^b$ | 416.7±18.6$^c$ | 230.8±10.8$^a$ | 72.8±6.8$^d$ |
| Change % | | +146* | -21** | -56** | -86** |
| Prolactin ng/dl | 48.8±3.2$^a$ | 92.3±5.3$^b$ | 68.9±3.8$^c$ | 33.7±3.4$^d$ | 10.3±1.6$^e$ |
| Change % | | +89* | -25** | -63** | -89** |
| r | + 0.97 | +0.093 | +0.90 | +0.94 | +0.93 |

Mean ±SE

a,b,c,d and e = Means in the same rows sharing the same subscript are not significant at $p \leq 0.05$

* = Change % in comparison to the control (1st group).

** = Change % in comparison to the $E_2$ (2nd group).

\+ = Increase in the change % or positive (r). - = Decrease in the change %

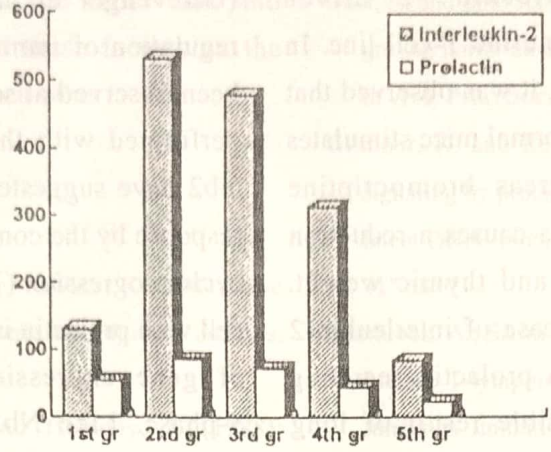


Fig. (1): Serum interleukin-2 (pg/dl) and prolactin (ng/dl) levels in immature male rats injected daily with estradiol benzoate in immature male rats injected daily with estradiol benzoate ($E_2$) for 10 days (2nd group) followed by injection of bromocriptine for 7 days at three different doses (3rd, 4th and 5th groups).

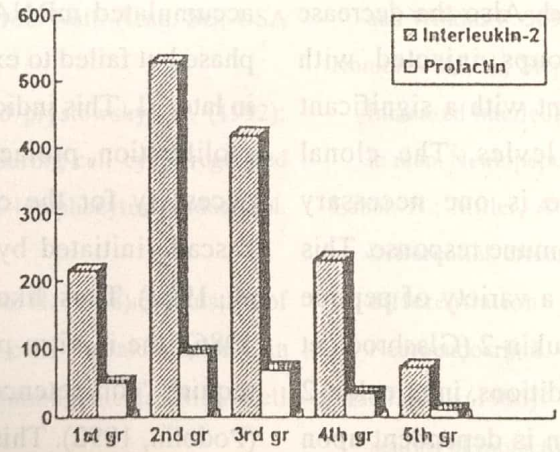


Fig. (2): Serum interleukin-2 (pg/dl) and prolactin (ng/dl) levels in immature male rats injected daily with estradiol benzoate in immature female rats injected daily with estradiol benzoate ($E_2$) for 10 days (2nd group) followed by injection of bromocriptine for 7 days at three different doses (3rd, 4th and 5th groups).

prolactin. Recently. Clevenger et al. (1990) demonstrated that prolactin is necessary but not sufficient for the interleukin -2 driven proliferation of the cloned murine T-cell line. In the study of Skwarlo (1992), it was observed that prolactin administration to normal mice stimulates antibody production, whereas bromocriptine -induced hypoprolactinaemia causes a reduction in both antibody response and thymic weight. However, a significant increase of interleukin-2 basal level in patients with prolactin secreting pituitary tumors as a possible result of long standing prolactin elevation (Komorowski et al., 1994).

The results of the present investigation revealed a positive correlation coefficient between prolactin and interleukin-2 serum levels which were not less than 0.9 in all animal groups (Tables 1 & 2). As the serum prolactin levels increased in animal groups injected with estradiol benzoate, the serum interleukin-2 levels increased. Also the decrease of prolactin levels in groups injected with bromocriptine was concurrent with a significant decrease in interleukin-2 levles. The clonal expansion of T-lymphocyte is one necessary component of an effective immune response. This proliferation is regulated by a variety of peptide growth factors sush as interleukin-2 (Glasbrook et al., 1981). Under in vitro conditions, interleukin-2 stimulated T-cell proliferation is dependent upon the presence of other peptides including transferrin (Neckers nd Cossman, 1983) and the neuroendocrine hormone prolactin (Clevenger et al., 1990). These peptides serve as progression factors, but not sufficient for T-lymphocyte proliferation. Removal of prolactin in vitro, through the use of antiprolactin antiserum inhibits the interleukin-2 driven proliferation of T-cells (Celevenger et al., 1992). The physiological regulation of immune response by prolactin has been observed at several levels. The experiments performed with the transformed rat T-cell line Nb2 have suggested that prolactin regulates this response by the control of genes necessary for cell cycle progression (Yu, 1990). Stimulation of Nb2 cell with prolactin is sufficient to induce a cascade of gene expression that permits entry into S-phase. Like Nb2 cells, the nontransformed murine T-cell line requires prolactin for proliferation (Clevenger et al., 1990). Unlike the Nb2 line, however, the murine T-cells require both interleukin-2 and prolactin before S-phase entry (Clevenger et al., 1992). In the absence of interleukin-2, prolactin stimulation of murine T-cells only induced interfeon regulatory factor-1 mRNA, but in the absence of prolactin, interleukin-2 stimulated murine T-cell accumulated mRNA expressed during early G1 phase but failed to express genes that are activated in late G1. This indicates that during cloned T-cell proliferation, prolactin serves as a growth factor necessary for the completion of an expression cascade initiated by interleukin-2 (Clevenger et al., 1992). Thus, like fibroblasts (Stern and Smith, 1986), the in vitro proliferation of cloned T-cells require "competence" and "progression" signals (Podolin, 1992). This model would suggest that an organized cascade of signals in necessary for in vivo T-cell activation and proliferation i. e. the G0 —>G1 transition and secretion of interleukin-2 is induced by antigen stimualtion, the early G1 —> late G1 transition is driven by interleukin-2, and the late G1—> S-phase transition by prolactin. It

would appear from the present investigation and that reported by Lahat et al. (1993), the synergism between interleukin-2 and prolactin could therefore be explained by the cross talk and mutual enhancing effect of these factors on the receptor concentration of each other

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