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Stimulation of Prolactin Synthesis by β- Glucan via Dectin-1 Receptors in GH3/B6 Cells

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Abstract: Prolactin (PRL) is a polypeptide hormone of the anterior pituitary gland that was originally named for its ability to promote lactation. In the present study, the mechanism of beta-glucan effect on GH3/B6 cells in the synthesis of prolactin has been investigated. In order to study the synthesis and secretion of prolactin, GH3 cell lines which is derived from pituitary tumors was used. Furthermore, the expressions of β-glucan dectin-1 receptors on these cells were studied. The intracellular prolactin levels were assayed by ELISA in the presence of 5, 150 and 250 µgr/ml of β-glucan for a period of 48 hours. TRH (50 nM) was used as positive control and the specific effect of beta-glucan was assessed by using dextran (150 µgr/ml). We also measured the expression of dectin-1 receptor in GH3/B6 cells. Our result showed that the higher concentrations of beta-glucan (150-250 ug/ml) significantly stimulate PRL synthesis. Most notably, for the first time we showed GH3/B6 cells express low levels of Dectin-1 receptor which greatly induced by beta glucan high concentrations. Therefore, β-glucan may induce PRL secretion throughout enhanced expression of Dectin-1 receptor.

Keywords: β-glucan, Dectin-1, GH3/B6 cells, Prolactin

1. Introduction

Prolactin (PRL), a protein hormone initially identified from the anterior pituitary that has deep effects on the mammary gland. PRL secretion is mediated through the dopaminergic pathways (Vera-Lastra, et al., 2002). Recently PRL has been shown to be a remarkably versatile hormone/cytokine with lots of biological actions in vertebrates (Nicol and Bern 1972, Goffin et al 2002) such as proliferation, differentiation and cell survival. The secretion of PRL is highly regulated by diverse neurotransmitters that act on the anterior pituitary. Its release can be stimulated by TRH through the activation of phospholipase C. Other neurotransmitters such as dopamine and acethylcholin suppress the release by inhibiting adenyl cyclase (Begonia.Y et al 2000). Numerous reviews indicate that PRL can have effects on the immune system (Yu-Lee et al 1997, Kooijman R et al 1996, maleral L 1997). It is now recognized that PRL is a cytokine which produces in some extrapituitary sites including neurons, prostate, decidua, mammary epithelium, endothelial cells, skin cells, and immune cells (Ben-Jonathan et al., 1996). PRL is important to maintain immune competence and plays an important role in animal and human immune responses (Yu-Lee et al., 2002).To study the synthesis, processing and secretion of prolactin at the cellular and molecular level, cell lines derived from pituitary tumors have been developed. GH3 cell line and its subclones secrete significant amounts of prolactin as well as low and variable amounts of growth hormone (GH) that are derived from rat anterior pituitary tumor (Bole-Feysot et al., 1998). Previous studies have shown,

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some hormones such as thyrotrophin releasing hormone (TRH) and plant extracts particularly polysaccharides such as glucans and pectin, can stimulate prolactin secretion from these cells (Sepehri et al., 1990; Ray and Willis, 1984).

Glucans are the major cell wall components of some bacteria, plants, both pathogenic and saprophytic fungi. They are polymers of glucose that can exist as a non-branched (1-3)-β-linked backbone or as the backbone with (1-6)-β-linked branches either in a single helix or triple helical conformation (Williams et al., 2003). Glucans are known as potent inducer of humeral and cell-mediated immunity (Lukas kubala et al 2003). The potentiation of host defense mechanisms has been suggested that glucans are possible means of tumor growth inhibition. The first step in modulation of glucans seams the binding to a specific cell surface receptor on the cells. As we previously showed that β-glucan derived from barely increase PRL secretion from pituitary explants, it seams that β-glucan may have specific receptor on pituitary cells. To reach this goal, we use GH3/B6 cells as model pituitary cells and investigate these cells to express a beta-glutan specific receptor.

2. Materials and Methods

2.1. Rat Somatomammotroph (GH3/B6) Cell Line

GH3/B6 cell line was a gift from Dr.D. Gordji (INRA, France). cells were cultured in Ham's F12 medium (HF12,Gibco.Invitrogene) supplemented with 15% horse serum(Hi Media, India) and 2.5% fetal calf serum (FBS,Hi Media, India). The cells were maintained in a humidified chamber with 95% air and 5% CO_2 at 37˚ C. The cells were grown to confluence and detached from monolayer with 0.025% trypsin. The cells used in the experimental were from fifth to ninth subcultures.

2.2. Isolation of GH3/B6 Cells

GH3/B6 cells were harvested plated into individual wells of 12-well multidishes containing 1.5 ml of complete medium (1 10^6 cells). Following equilibration (24 hours), cells were treated with HF12 contained barely β-glucan (sigma, USA) at final concentrations of 0, 5, 150, and 250 g/ml for 48 hours. β-glucan solution was prepared in water/ethanol a ratio of 94-6. TRH (50 nM) was employed as the positive control (Ray and Willis, 1984) and dextran (150 g/ml) was used as the negative control.

2.3. Prolactin Secretion from GH3/B6 Cells

After 48 hours, cells were washed in phosphate-buffer saline (PBS) and viable cells counted using trypan blue. Whole cell lysates of GH3/B6 cells were prepared by lysis buffer (0.5%NP_{40}, 1.5 mM $MgCl_2$, 0.14 M NaCl, 10 µM Tris-HCl, and 100 µgr/ml phenyl methyl sulfonyl (PMSF)). The cell lysate was saved for sandwich enzyme immune assay using a rat ELISA kit (MD biosciences, USA).

2.4. Total RNA Extraction

Total RNA was extracted from GH3/B6 cells and spleen using TRIzol reagent (Invitrogen, CA) according to to the method described by Comczynski and Sacchi (Chomczynski and Sacchi, 1987). The purity and concentration of the RNA was measured using spectrophotometer, and an optical density 260/280 ratio of greater than 1.9 was considered a pure sample.

2.5. Polymerase Chain Reaction

To produce a first strand cDNA copy of the RNA, 10 µg of the total RNA was was mixed in a 0.2 ml PCR tube with 1 µl Random Hexamer (Fermentase) and incubated for 5 min at 70ºC. The tube was placed on ice and 4 µl First-Strand Buffer, 2.5 µl dNTP ND 1 µl RNAse inhibitor added. This solution was mixed and incubated for 5 min at room

temperature. 1 µl of revers transcriptase (fermentase) was added and incubated for 1 hour at 42 ºC followed by inactivation by incubating for 10 min at 70 ºC. 1µl of the product was used for amplification of gene products using a PCR protocol described below. The reaction mixture was made of specific oligonucleotide primers (20 Pmol), dNTP (0.2 mmol) and Tag DNA polymerase (5 Unit/µlit) (Fermentose, Lietuva). Then this mixture was added to the RT product.

In the set of amplification, because of low expression of Dectin-1 gene, 40 cycles was used. denaturation temperature was 94 ºC for 5min and annealing temperatures were 94ºC for 1min, 56 ºC for 2min and 72 ºC for 3min. Reaction products were electrophoresed on 6% polyacrylamide gel. Each PCR reaction included 18S rRNA gene used as internal control.

For the detection of the rat Dectin-1, we design oligonucleotide primer pairs based on the sequence of predicted mRNA sequence of rat Dectin-1 and the high homology between rat and mouse genomes. The set of primers are as followed: 3' GGA TGG ATC AGC ATT TAC CC 5' (forward) and 3'TCT GCA TCC AAC TTC TCA GC 5' (reverse). The predicted size of amplified product was 340 bp.

2.6. Data Analysis

The data are expressed as means ± S.E.M. The statistical analyses were performed using one-way analysis of variance (ANOVA). Following a significant F-value, post-hoc analysis (Tukey) was performed for assessing specific group comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Cells Characteristics

Fig. 1 shows the viability of GH3/B6 cells in the presence of the different concentrations of -glucan via trypan blue exclusion test. The different concentrations of -glucan, TRH and dextran do not affect the viability of these cells in a 48-priod [$F(6, 30) = 24$, $p > 0.05$].

3.2. β-glucan Increases Intracellular Prolactin in GH3/B6 Cells

Fig. 2 shows the effect of β-glucan on prolactin secretion in GH3/B6 cells. Incubation of pituitary somatommamotroph cells (GH3/B6 cells) with β-glucan for 48 hours changed the intracellular prolactin levels [$F(5,19)$, $P < 0.0001$]. Post-hoc analysis indicated that the higher doses of β-glucan (150 and 250 µg/ml) increased intracellular prolactin significantly in comparison to the control groups which treated in the media and dextran. This elevated level in concentration of 150 µg/ml of β-glucan is comparable to TRH(Thyrotropine Releasing Hormone). TRH is a well known prolactin stimulator in GH3/B6 cells. β-glucan concentration of In 250 µg/ml induced high amount of prolactin secretion. Prolactin levels were not changed significantly in low concentration of β-glucan (5 µg/ml). Dextran, the negative control, didn't stimulate intracellular prolactin. Therefore, β-glucan high concentrations stimulate prolactin synthesis in GH3/B6 cells. There appears to be a specific effect specific effect as dextran has no effect on intracellular prolactin level.

3.3. β-glucan Increase of Dectin-1 Expression in GH3/B6 Cells

Quality of mRNA was shown by integrity of 18s and 28s bands in Fig. 3(a). Specific sense and antisense oligonucleotide primers based on the sequence of the rat Dectin-1 gene were used to amplify. The PCR- amplified products from the experiments were run on acrylamide gel and investigated. As shown in fig. 3(b) our positive control, spleen cells, expressed Dectin-1 strongly. GH3/B6 cells without any treatments expressed Dectin-1 at very low levels.

Adding β-glucan in the cells culture media caused Dectin-1 levels increase in these cells. This result also shows the expression of Dectin-1 in rat splenocytes. It is the first report of Dectin-1 presence in rat cells.

4. Discussion

In the present study, we observed that intracellular prolactin increase in GH3/B6 cells in the presence of β-glucan. It seems this effect is done through Dectin-1 receptor.

It has been reported that cell wall polysaccharide of plants such glucan and pectin have lactogenic properties. The intravenous injection of -glucan caused the stimulation of prolactin secretion (Carlson et al,. 1985). Our previous study indicating that -glucan stimulated prolactin secretion in ewe pituitary fragments and GH3/B6 cells (Sepehri et al., 1990; Delphi et al., 2006). In the present study, we showed that incubation of GH3/B6 cells with -glucan with in concentration also increases intracellular prolactin significantly after 48 hours. Therefore, it seems -glucan could induce prolactin synthesis in GH3/B6 cells.

As β-glucan could stimulate prolactin synthesis and secretion, it must have specific receptor on these cells to stimulate them. Breuel et al. have reported the binding of -glucan to GH3/B6 membrane is saturable, dose-dependent and specific (Breuel et al., 2004). Any effect of dextran on intracellular prolactin confirms these data, since dextran another polymer of glucose did not bind to GH3/B6 cells and has no specific effect on GH3/B6 cells. Thus, in the case of GH3/B6 cells, it appears that the -glucan should be cross-linking a single type of receptor though this interaction is sufficient to induce prolactin release.

Glucans are immunostimulator compounds and act via specific receptor. The nature of glucan receptors is currently under investigation. These receptor that are expressed in immune and non-immune tissue are complement receptor 3 (CR3), lactosylceramide, scavenger receptors and dectin-1 (Breuel et al., 2004; Brown and Gordon, 2001)

CR3 is a β2 integrin which is leukocyte-restricted, thus it would not be found on other cells. Brown, Gordon and Willment et al. have recently identified Dectin-1 as the primary β-glucan receptor (Breuel et al., 2004). Dectin-1 is a pattern recognition receptor (PRR) that recognizes Pathogen Associated Molecular Patterns (PAMP) on microbes. Carbohydrates, lipids, proteins and nucleic acid are major PAMPs. Dectin-1 receptor expresses in macrophages, monocytes, neutrophils and subset of T cells and many non-immune tissues (Gordon , 2006). Dendretic cells which express Dectin-1 at high levels found in spleen, so we have used spleen as positive control. As we expected spleen cells express Dectin-1 receptor in high level. Expression of dectin-1 receptor in GH3/B6 cells increase under effect of -glucan. So, -glucan stimulates these cells via dectin-1 receptor and cause elevation both prolactin synthesis and secretion.

As previously mentioned, prolactin hormone has variant role in immune system. It can stimulate T cells, B cells, natural killer (NK) cells, macrophages, neutrophils, $CD34^+$ hematopoietic cells and antigen-presenting dendritic cells in immune system (Dogusan, 2001). Interestingly it is known that elevated PRL levels have been reported in patients with autoimmune diseases such as Systemic lupus erythematosus (SLE or lupus), multiple sclerosis, rheumatoid arthritis, psoriatic arthritis, AIDS and patients prior to transplant rejection (Kanik and Wilder, 2000). In the hematopoietic system, prolactin enhances GM-CSF-mediated maturation of $CD34^+$ human hematopoietic progenitor cells into erythroid precursors in culture. Pharmacologic levels of PRL increases the hematopoietic progenitors of the myeloid (CFU-GM) and erythroid (BFU-E) lineages in the bone marrow and spleen, during myelosuppression following treatment for HIV infection or bone marrow transplantation (Richards and Murphy, 2000). PRL and estrogen have been shown to be protective against inflammation in the context of severe trauma (Yu-Lee et al., 2002).

According to the roles of prolactin in immune system, we can assume -glucan, a major PAMP of bacteria and fungi cell wall with immunostimulatory activity stimulates prolactin synthesis and secretion from anterior pituitary lactotroph cells via Dectin-1 receptor, and consequently prolactin activate another immune reactions. All these reactions and connections help hemostasis maintenance. It needs more studies to mention whether or not Dectin-1 in GH3/B6 cells intermediates through activation of intracellular cascades such as immune cells.

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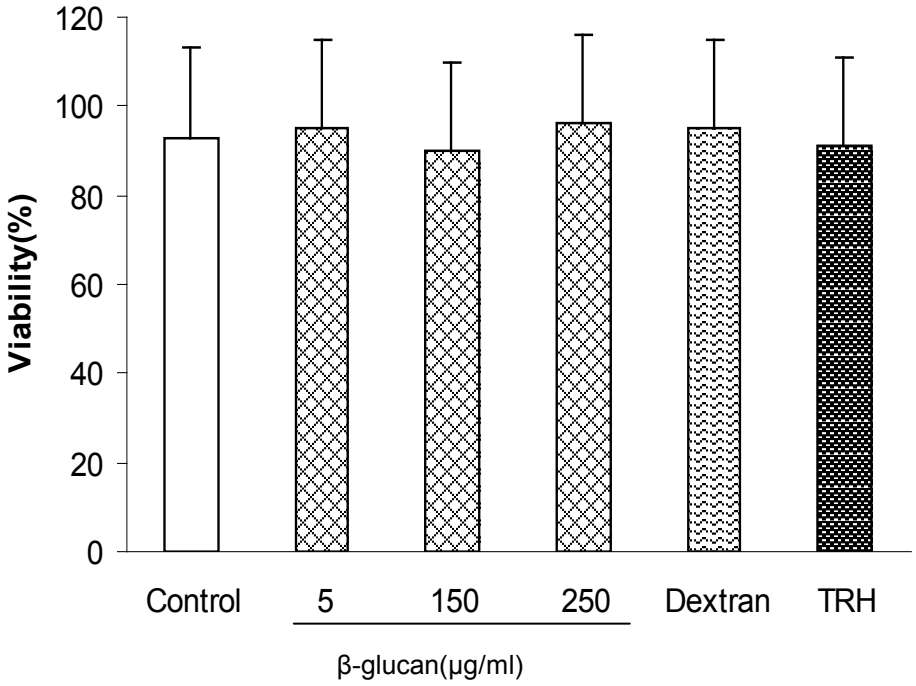


Fig. 1. Effect of β-glucan, TRH and dextran on GH3/B6 cell viability. GH3/B6 cells were treated for a period of 48 by the different concentrations of β-glucan (0-250 μg/ml), TRH (50 nM) and dextran (150μg/ml). The results are expressed in the viability percentage (n = 5).

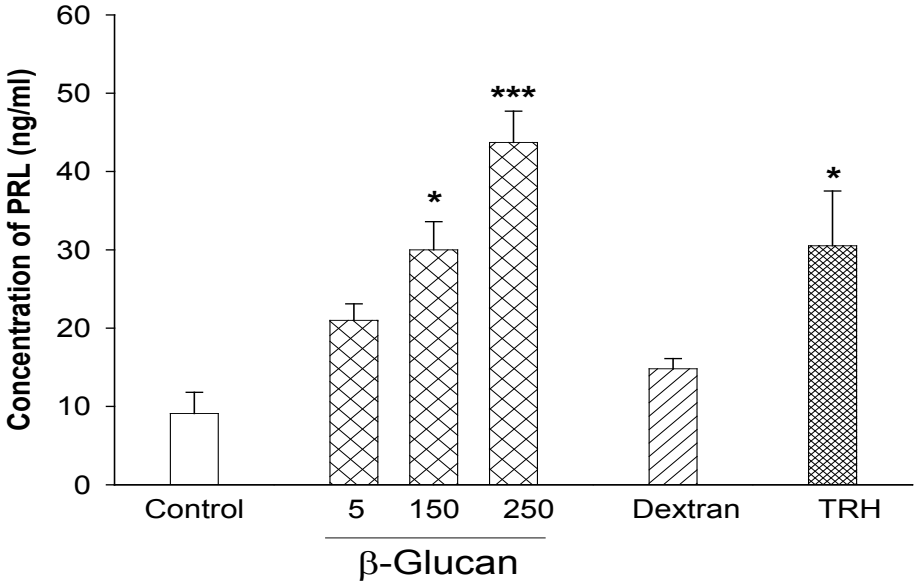
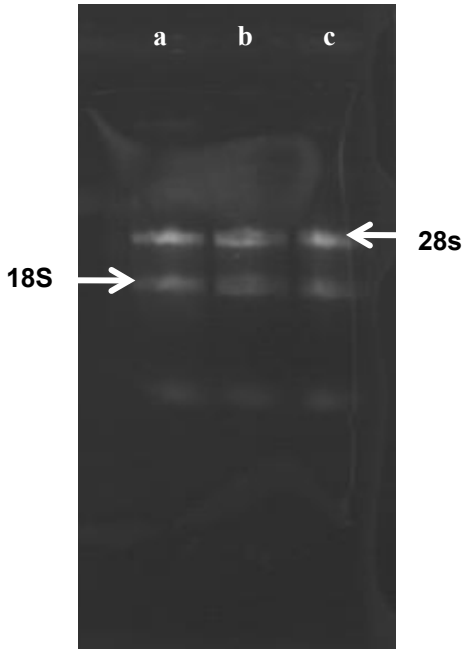
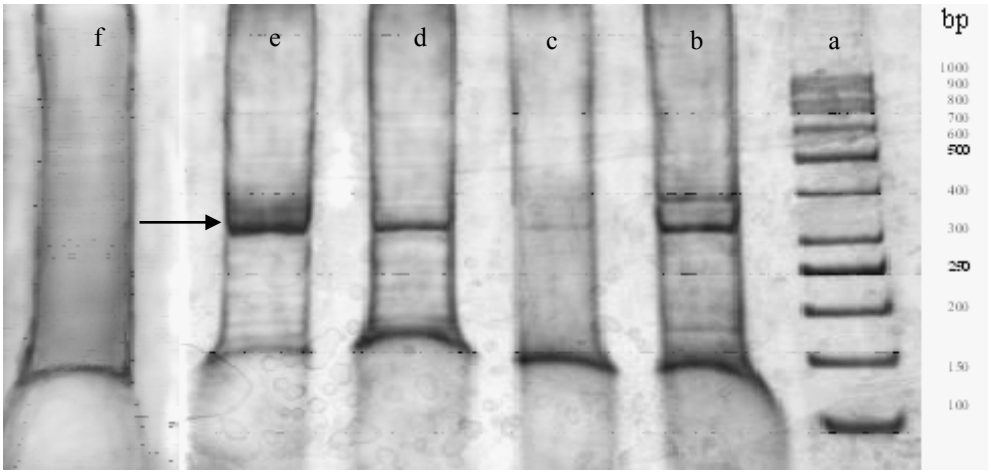


Fig. 2. Comparison of intracellular levels of prolactin in GH3/B6 cells under effect of different concentrations of -glucan (0-250 g/ml), TRH (50 nM) and dextran (150 g/ml) * P<0.05, *** P<0.001) n =3).



(a)



(b)

Fig. 3 **(a).** RNA Agarose gel:

a) GH3/B6 cells RNA under effect of β-glucan (250 μg/ml)
b) GH3/B6 cells RNA
c) Spleen cells RNA

(b). PCR production on acryl amid gel:

a) ladder
b) spleen cells
c) GH3/B6 cells
d) GH3/B6 cells under effect of β-glucan (250 μg/ml)
e) 18S
f) Dectin-1 primer