Iranian Journal of Science & Technology, Transaction A, Vol. 31, No. A3
Printed in The Islamic Republic of Iran, 2007

THE EFFECT OF β-GLUCAN ON PROLACTIN SECRETION IN GH3/B6 CELLS*

H. SEPEHRI**, L. DELPHI AND Y. RASSOULI

Animal Physiology Department, School of Biology, College of Science, University of Tehran, I. R. of Iran
Email: hsepehri@khayam.ut.ac.ir

Abstract – It is well known that several plant extracts have a lactogenic effect. This response may be induced through the stimulation of prolactin release from lactotrope cells. A partial purification of the active compound indicated that it is rich in pectin and β-glucan. These materials stimulate secretion of prolactin from hypophyseal fragments. In the present study, the effect of β-Glucan on the secretion of prolactin from GH3/B6 pituitary tumor cells has been investigated. These cells are good tools for studying the direct effect of β-glucan on prolactin secretion. The different concentrations of β-glucan (50, 100 and 200 μg/ml) increased secretion of prolactin within 24 hours of incubation in GH3/B6 media. Moreover, the secretion of prolactin was significantly elicited at 100 and 200 μg/ml of β-glucan incubation after 48 hours. These results suggest that β-glucan can affect the synthesis and/or release of prolactin, a hormone that plays many important physiological roles from GH3/B6 pituitary tumor cells.

Keywords – Lactotropes, prolactin, GH3/B6 cells, β-glucan

1. INTRODUCTION

Rat pituitary cells consist of somatotropes (GH^+/Prl^-), lactotropes (GH^-/Prl^+), and lactosomatotropes (GH^+/Prl^+). The lactotropes secrete prolactin, primarily. The rat pituitary cell line GH3 and its subclones secrete significant amounts of prolactin as well as low and variable amounts of growth hormone (GH) [1]. These cells have been widely used to study the mode of action of several hormones that regulate prolactin secretion *in vivo*. These include neuropeptides such as thyroliberin, vasoactive intestinal peptide, thyroid hormones, and estrogens [2]. It is important to note that GH3/B6 cells secrete prolactin and growth hormone (GH) in a ratio of 5-1 [3].

Plant extracts are widely used to stimulate the various physiological functions. Some plant extracts stimulate secretion of prolactin, including beer which increases milk secretion. Originally, it has been suggested that these effects were due to the alcohol part of beer. However, it has been reported that the plant components of beer were responsible for this effect [4]. The extracts from hops were not active, whereas malt and barley from beer strongly stimulated prolactin secretion. Considering the lactogenic agents were water-soluble and resistant to heat or proteases, it seems that they are polysaccharides. Chemical analysis of these extracts revealed that the major parts of the polysaccharides were pectin and β-glucan [5, 6]. Further studies confirmed that the strong capacity of these agents elicits prolactin secretion in experimental animals [7, 8] and the culture of pituitary fragments [9].

β-glucan is a heterogeneous group of glucose polymers, consisting of a backbone of β1→3 linked β-D-glucopyranosyl units with β1→6 link side chains of varying distribution and length. These substances

*Received by the editor May 6, 2006 and in final revised form October 27, 2007
**Corresponding author

were produced as cell wall constituents of fungi, algae, lichens, and plants [10]. The compounds have anti-infective and anti-tumorigenic properties [11]. However, the structural variability of polysaccharides obtained from the various sources influence their biological activity. Some investigators suggest that the parameters such as the primary structure, degree of branching, solubility, molecular weight and polymer charge may influence β-glucan activity [12].

In order to determine the exact mechanism of these agents at cellular and molecular levels, the studies on their *in vitro* effects is essential. For this purpose, the effect of β-Glucan on the secretion of prolactin from GH3/B6, a subclone of anterior pituitary gland tumor pituitary cells, were investigated using the western blotting technique. In addition, we studied morphological changes of the cells after β-glucan treatment.

2. MATERIALS AND METHODS

a) Cell culture

GH3\B6 cells were grown as monolayer in Ham's F12 medium (Hi Media, India) enriched with 15% heat inactivated horse serum (Hi Media, India), 2.5% fetal calf serum (Hi Media, India), 50 IU/ml penicillin and 50 μg/ml streptomycin and maintained at 37˚ C in an atmosphere of 95% air and 5% CO_2 [13]. The GH3\B6 cells were from the fifth and ninth subcultures.

GH3/B6 cells were seeded at 1×10^6 cells/ml in complete medium within 6 well plates. After 3 days the culture medium was replaced with a new one containing β-glucan at final concentrations of 0, 50, 100, 200, and 300 μg/ml for 24 and 48 h, after which the conditioned media were collected for further analysis. Thyrotropin releasing hormone (TRH) was used as (50 nM) positive control [14].

b) Preparation of Rabbit anti-rat Prolactin

Polyclonal antibody against prolactin was prepared in New Zealand White rabbits (1.5-1.8 Kg) using purified rat prolactin as the antigen. The rat prolactin emulsified in the same volume of the Freund's incomplete adjuvant (Sigma). On the first day, 0.2 ml of this emulsion was injected to the rabbits. After 2 days, the animals received 0.5 ml of the emulsion again. All animals then received once weekly injections of the emulsion (1 ml/rabbit) for six weeks. After the 3rd week, blood samples (1.5 ml/rabbit) were collected by bleeding rabbits from the marginal vein of their ear. The collection of blood samples continued until the 7^{th} week. The samples were placed at 37°C for 1 h to inactivate complement, and were at 4°C overnight to clot. The blood serum was separated with centrifuge at 4°C, 10000 g for 20 minutes. This serum was used as the first antibody in the blotting procedure [15].

c) Western Blot Analysis

Western blot was carried out for quantitative analysis of prolactin in conditioned media in the presence of β-glucan. The conditioned media containing prolactin were mixed with the same volume of sample buffer (50 nM Tris-HCl buffer pH= 6.8, dithiothriltol, 2% SDS, 10% glycerol and 0.1% bromophenol blue), and heated at 95°C for 8-10 min. The samples were resolved on 12% SDS polyacrylamide gel using 25 mM Tris-glycine buffer, pH= 8.3 at 160 mV for 2 h at room temperature. The proteins were separated by the SDS-PAGE and then transferred to nitrocellulose membrane using 25 mM Tris-glycine buffer, pH= 8.3, containing 20% methanol. The electrical parameter was set at 0.65 mA per gel surface. The complete transfer of the proteins from the gel to the membrane was confirmed by staining the gel with Coomasie Blue after electrophoretic transfer. The membranes were: (i) washed 4 times in TBS (20 mM Tris-HCl buffer pH= 7.4 and 0.9% NaCl); (ii) incubated in 5% bovine albumin (Sigma) for 2 h at room temperature with gentle shaking; (iii) incubated with the primary antiserum at the dilution of

1:100 overnight; (iv) washed again with TBS 3 times for 5 min; (v) incubated with anti-rabbit IgG HRP conjugated antibody (Sigma) at a dilution of 1:1000 for 2 h; (vi) washed again with TBS; and (vii) were treated with the substrate (4-choloro 1-naphtol, Sigma) until the color-bands were developed. The reaction was terminated by soaking the membrane in double distilled water [15]. Prolactin bands on the developed blots were digitally captured with a UVI Tec and band intensities were measured using Totallab® software, a software for 1D electrophoresis gel analysis, comparison and data-minin. (Nonlinear Dynamics Ltd. USA).

d) DNA extraction

The cells were treated with proteinase K and the DNA was precipitated with isopropanon and dissolved in TE solvent. The optical densities were read at 260 and 280 nm [16].

e) Statistical 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. Differences with $p<0.05$ between experimental groups at each point were considered statistically significant.

3. RESULTS

a) Cell proliferation

The viability and growth of GH3/B6 cells were determined in the presence of the different concentrations of β-glucan via the thrypan blue exclusion test. As shown in Fig. 1, one-way ANOVA revealed that β-glucan had no significant effect on the viability of GH3/B6 cells in incubation of 24 h [$F(5, 12) = 3.7$, $P > 0.05$] and 48 h [$F(5, 12) = 1.259$, $P > 0.4$]. Indeed, the addition of TRH did not change the cell viability. Significant change was not observed in the cells' DNA content in the presence of β-glucan in incubation of 24 h [$F(5, 12) = 1.6$, $P > 0.05$] and 48 h [$F(5,12) = 0.054$, $P > 0.05$] (Table 1).

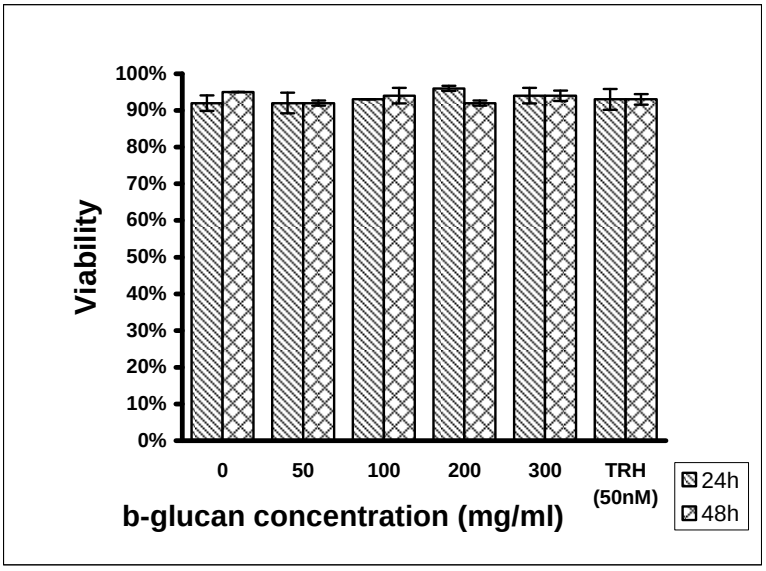

Fig. 1. Effect of β-glucan and TRH on GH3/B6 cell viability.GH3/B6 cells were treated for a period of 24h and 48h by the different concentrations of β-glucan (0-300 μg/ml) and TRH (50 nM). The results are expressed in the viability percentage (n=3)

Table 1. Effects of β-glucan on GH3/B6 DNA content (Mean ± SEM, n=3)

β-glucan concentration (μg\ml)	**DNA content (mg/ml)**	
	24h	**48h**
0	0.285± 0.09	0.509± 0.16
50	0.231± 0.06	0.310± 0.04
100	0.237± 0.06	0.502± 0.19
200	0.292± 0.03	0.420± 0.08
300	0.283± 0.01	0.397± 0.23
TRH (50nM)	0.268± 0.005	0.546± 0.08

b) Cell attachment and morphology

GH3/B6 cells attached and spread onto tissue culture dishes in the serum supplemented medium (Fig. 2a). In the presence of TRH, the cells attached to the culture dishes (Fig. 2b). Furthermore, 24 h incubation with β-glucan caused the cells to become spherical and small vesicles appeared in their cytoplasm, indicating β-glucan affected the cell morphology (Figs. 3a and 3b). The decrease in cells spreading was observed after 48 h (Fig. 3c and 3d). In 48 h incubation, the number of vesicles was more than the 24h. In these conditions, GH3/B6 cells attached to the culture dishes.

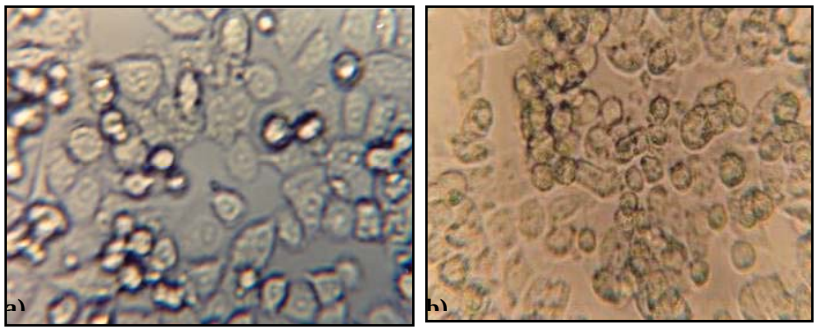

Fig. 2. (a) GH3/B6 cells in a serum supplemented medium, (b) GH3/B6 cells in the presence of TRH (50 nM)(25X)

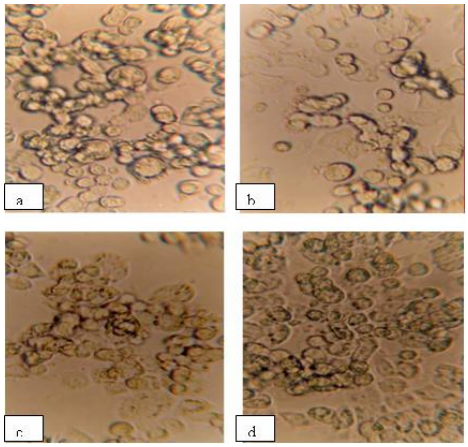

Fig. 3. GH3/B6 cell morphology: (a) 50 μg/ml (b) 200 μg/ml of β-glucan treatment after 24 h, (c) 50 μg/ml (d) 200 μg/ml of β-glucan treatment after 48 h (25X)

c) Effect of β-glucan on prolactin secretion

Figs. 4 and 5 show the effect of the addition of β-glucan in the cells media on the secretion of prolactin. One-way ANOVA revealed that β-glucan caused a significant secretion of prolactin in 24 h [$F(5,18) = 5.83$, $P<0.01$] and 48 h [$F(5,18) = 4.86$, $p<0.01$] incubation. Post-hoc analysis indicated that the maximum effect of β-glucan on prolactin secretion was produced by concentrations of 50 ($P<0.01$) and 100 μg/ml ($P<0.01$) in 24 h incubation, while 200 μg/ml of β-glucan was more effective in 48 h incubation ($P<0.01$). In both groups, β-glucan was more effective in prolactin release as compared with TRH.

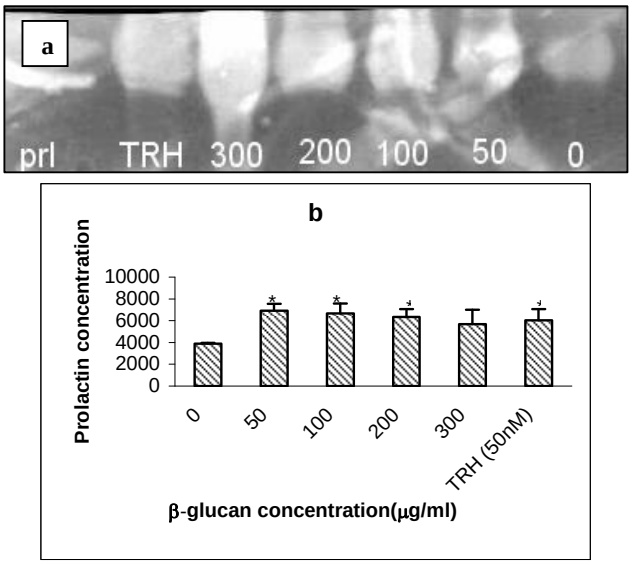

Fig. 4. (a) Prolactin bands were transferred onto nitrocellulose membrane and subjected to scanning followed by immunoblotting. (b) Effects of β-glucan on prolactin secretion after 24 h incubation.
Data are expressed as mean ± SEM (*<0.05, **<0.01)

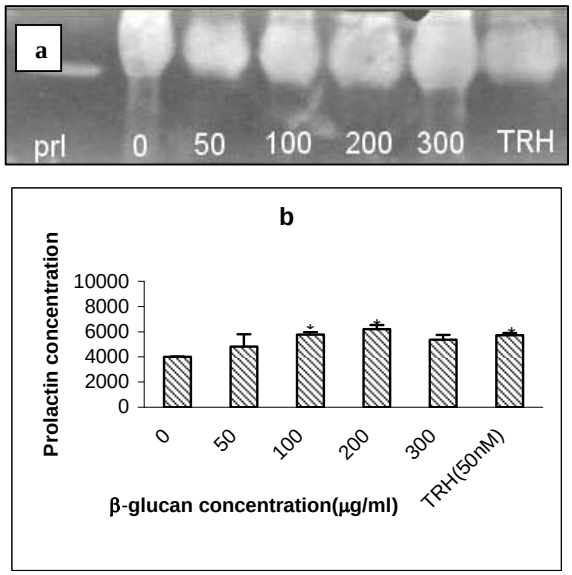

Fig. 5. (a) Prolactin bands were transferred onto nitrocellulose membrane and subjected to scanning followed by immunoblotting. (b) Effect of β-glucan on prolactin secretion in 48 h incubation.
Values are expressed in mean ± SEM (*<0.05, **<0.01)

4. DISCUSSION

In the present study, we demonstrated for the first time, the effect of β-glucan on prolactin secretion from GH3/B6 cells. Our data indicated that co-incubation of GH3/B6 cells with β-glucan stimulated prolactin secretion. This may be supported by our previous study indicating that β-glucan stimulated prolactin secretion from ewe hypophysis fragments (7, 8). The present data also showed that β-glucan is able to affect specifically on rat lactosomatotropes. It has been reported that the intravenous injection of β-glucan led to the similar stimulation of prolactin secretion (4). The rat lactosomatotropes can express single glucan pattern recognition.

In the present study, the results revealed that β-glucan may be able to change the functional state of these cells through its receptors. It seems that the stimulation of β-glucan receptors elicits prolactin release. In accordance with this hypothesis, Breuel and coworkers showed that glucan must be cross-linking a single type of receptor and that this interaction is sufficient to induce prolactin release in somatomammotrophs [17]. It is important to note that β-glucan receptors are not precisely described, but recent investigations suggest that β-glucan act through complement receptor 3 (CR3), lactosylceramide, scavenger receptors and dectin-1 [18, 19]. Glucans pattern recognition receptors have been identified on macrophages, neutrophils, NK cells, T cells, dentritic cells, fibroblasts, microvascular endothelial cells, and anterior pituitary cells [18, 20]. Dectin-1 was suggested to be an important receptor for the β-glucan receptor which recognizes carbohydrate containing β-1,3 or β-1,6 glucan linkage and is expressed on most immune cells [21]. However, there is no report of dectin-1 expression in the pituitary cells. Although the mechanisms by which glucans modulate their biological activities have not been elucidated, some observations indicate that highly purified glucans stimulate the phosphoinositide-3-kinase pathway, which is a negative feedback mechanism for proinflammatory responses (12).

In the present experiment, β-glucan stimulated prolactin secretion after 24 and 48 h incubation. This response may show the stability of this substance in the culture medium, which leads to prolactin synthesis. In agreement with this result, previous study has shown that the systemic administration of various glucan preparations stimulated the release of prolactin in sheep and cows (8). Moreover, it is reported that β-glucan stimulated prolactin secretion from hypophyseal fragments (9, 10). Although the data did not suggest β-glucan mechanism of action, there is a report that suggests β-glucan may be acting indirectly (8). To clarify the exact mechanisms underlying the above interactions, more studies are required.

In conclusion, our results show β-glucan can affect the synthesis and release of prolactin from GH3/B6 pituitary tumor cells. The increase of prolactin secretion was observed in long-term incubation and the response supported by morphological changes.

Acknowledgements-This study was support by a research grant from the University Colleges of Science of the University of Tehran. We would like to express thanks to Dr. D. Gourdji and Dr. D. Houdebine of the INRA, France, and Dr. Golliae and Dr. A. Rezoyf for their skillful assistance.

REFERENCES

1. Glassmeier, G., Hauber, M., Wulfsen, I., Weinsberg, F., Bauer, C. K. & Schwarz, J. R. (2001). Ca^{++} channels in clonal rat anterior pituitary cells (GH3/B6). *Journal of Pflugers Arch, 442*(4), 577-587.
2. Gourdji, D., Tougard, C. & Tixier-Vidal, A. (1982). Clonal prolactin strains as a tool in neuroendocrinology. *Frontiers in Neuroendocrinology*, *7*, 317-351.
3. Gourdji, D., Kerdelhue, B. & Tixier-Vidal, A. (1972). Ultra structure d' un clone de cellules hypophysaires secretant de la prolactin (clone GH3). *Les Comptes rendus de l'Académie des sciences*, 437-490.

4. Carlson, H. E., Wasser, H. L. & Reidelberger, R. D. (1985). Beer induces prolactin secretion: a clinical and laboratory study of the role of salsolinol. *J Clinical Endocrinology and Metabolism, 60*, 677-701.
5. Sawadogo, L. & Houdebine, L. M. (1988). Identification of the lactogenic compound present in beer. *Annales de biologie clinique, 46,* 126-134.
6. Sawadogo, L., Houdebine, L. M., Thibault, J. F., Rouau, X. & Ollivier-Bousquet, M. (1988). Effects of pectic substance on prolactin and growth hormone secretion in the ewe and on the induction of casein synthesis in rat. *Reproduction Nutrition Development, 28* (2A), 293-301.
7. Sawadogo, L., Sepehri, H. & Houdebine, L. M. (1989). Evidence for a stimulating factor of prolactin and growth hormone secretion present in brewery draff. *Reproduction Nutrition Development, 29*, 139-146.
8. Sepehri, H., Renard, C. & Houdebine, L. M. (1990). β-glucan and pectin derivatives stimulate prolactin secretion from hypophysis in vitro. *Proceeding of the Society for Experimental Biology and Medicine* (193-197).
9. Sepehri, H., Zoraghi, R. & Haeri- Rohani, A. (2000). Effect of pectic acid and β-glucan on prolactin secretion by ovine pituitary explants. *Iranian Journal of Science, 1*, 99-107.
10. Ross, D.G., Vetivicka, V., Yan, J., Xia, Y. & Vetvickova, J. (1999). Therapeutic intervention with complement and β-glucan in cancer. *Immunopharmacology, 42,* 61-74.
11. Brown, D. G. & Gordon, S. (2003). Fungal β-glucans and mammalian immunity. *Immunity; 19*, 311-315.
12. Kubala, L., Ruzickava, J., Nickova, K., Sandula, J., Ciz, M. & Lojek, A. (2003). The effect of (1→3)β-D-glucan, carboxymethylglucan and schizophyllan on human leukocytes in vitro. *Carbohydrate Research, 338*, 2835-2840.
13. Gourdji, D., Tougard, C. & Tixier-Vidal, A. (1982). Clonal prolactin strains as a tool in neuroendocrinology. *Frontiers in Neuroendocrinology, 7*, 317-351.
14. Ray, K. P. & Willis, M. (1984). Studies of TRH-induced prolactin and its inhibition by dopamine, using ovine pituitary cells. *Mollecular Cellular Endocrinology, 36*, 131-139.
15. Yuan, X. H., Ju, Y. G., Lu, Sh., Yan, C., Zu, C. Z. & Yong, L. Z. (2002). Get effective polyclonal antisera in one month. *Cell Research, 12*, 157-160.
16. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual *(3 Volume Set),* 2nd edition. *Cold Spring Harbor Laboratory Press*.
17. Breuel, K. F., Rice, P. K., Duo, P. J., De Ponti, W., Wang, K., Laffan, J., Li, J., Kalbfleisch, J. & Williams, D. L. (2004). Anterior Pituitary Cells Express Pattern Recognition Receptors for Fungal Glucans: Implications for Neuroendocrine Immune Involvement in Response to Fungal Infections. *Neuroimmunomodulation, 11*, 1-9.
18. Lowe, E., Rice, P., Ha, T., Li, C., Kelley, J., Ensley, H. (2001). A (1→3)β-D linked hepatosaccharide is the unit ligand for glucan pattern recognition receptors on human monocytes. *Microbes Infection, 3*, 789-797.
19. Michalak, M., Melican, D., Brunke-Resse, D., Langevin, M., Lemerise, K. & Galbraith, W. (1998). Activation of rat macrophage by betafection PPG-glucan requires cross-linking of membrane receptors distinct from complement receptors 3 (CF3). *Journal of Leukocyte Biology, 64*, 337-344.
20. Gonzales, J. A., Digby, J. D., Rice, P. J., Breuel, K. F., DePonti, W. K., Kalbfeisch, J. H., Browder, I. W. & Williams, D. L. (2004). At low serum β-glucan concentrations there is an inverse correlation between serum β-glucan and serum cytokine levels. *International Immunopharmocology, 4*, 1107-1115.
21. Brown, D. G. & Gordon, S. (2001). A new receptor for β-glucan. *Nature, 413*, 36-37.