Effect of Pectic Acid and ?Ì-Glucan on Prolactin Secretion by Ovine Pituitary Explants

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Abstract

Recent studies have indicated that several plant extracts reputed to be lactogenic are capable of inducing -casein synthesis in mammary epithelial cells. This enhancement is mediated through a stimulation of prolactin release from lactotropes in anterior pituitary. Therefore it is admitted that these extracts are capable of stimulating prolactin secretion from hypophysis. A partial purification of these extracts, revealed that their active compounds are pectin in most cases and -glucan in other cases. In this work, the effect of various concentrations of both pure pectic acid and -glucan on prolactin secretion from ewe hypophysis fragments is investigated. It is shown that pectic acid in the 50-150 ?g*/ml* range concentration and -glucan in the 200-400 ?g*/ml* range concentration are capable of stimulating prolactin secretion significantly ($P<0.05$, $P<0.01$), from hypophysis explants.

Keywords: *Pectic Acid, ?Ì-Glucan, Prolactin Secretion, Ovine Pituitary Explants*

Introduction

Plant extracts are traditionally used in various societies for their pharmacological properties. Some plants which are reputed to be lactogenic have the capacity to enhance milk secretion in lactating women. Recent studies in this field have indicated that these extracts induced -casein synthesis in rat mammary gland *in vivo* (Sawadogo & Houdebine, 1988; Sawadogo *et al.*, 1988). It was observed that this effect is mediated through an effect on prolactin (PRL) secretion (Sawadogo & Houdebine, 1988) (Sawadogo & Houdebine, 1988; Sawadogo & Houdebine, 1989). The extracts proved to stimulate secretion of growth hormone and cortisol in addition to PRL, *in vivo* (Sawadogo & Houdebine, 1988). It is admitted that some plants in *Malvaceae, Linaceae, Euphorbiaceae* and *Umbelliferae* families have

the lactogenic capacity (Sawadogo & Houdebine, 1988). Chemical analysis of these extracts revealed that active compounds are polysaccharides rich in pectins in most cases and rich in -glucan in other cases (Sawadogo & Houdebine, 1988,1989).

Both pure pectic acid and -glucan showed a strong capacity to trigger PRL and GH secretion in experimental animals when injected intravenously (Sawadogo & Houdebine, 1988). From these data, it was concluded that pectic substances and -glucan are the active lactogenic compounds existing in plants, and that their effects are mediated through the secretion of PRL and possibly of GH and cortisol. *In vitro* experiments have shown that pectic acid is able to induce the secretion of PRL, GH, LH and -endorphin from rat hypophysis (Sawadogo, 1988). Moreover, this compound was shown to stimulate -casein secretion from rabbit mammary gland *in* (Sawadogo & Houdebine, 1988). The mechanism of action of the plant extracts is not yet known. Whether the lactogenic substances stimulate PRL secretion *in vivo* through a direct action on pituitary cells or through an indirect effect on hypothalamus hypophysis axis is not known. To get an answer for this question, the effect of various concentrations of lactogenic substances on PRL secretion from incubated ewe hypophysis fragments was investigated. Results confirm those previously obtained in a preliminary experiment (Sepehri *et al.*,1990). In this study, the optimal concentrations of lactogenic substances which have maximal stimulatory effects were determined.

Materials and Methods

Chemical Pectic acid (poly-D-galacturonic acid) was from Fluka and -glucan from Sigma. These materials were dissolved in 0.7% NaCl (pH=7.8) after stirring for 1 hour at 30°C. The pH of these solutions (1 mg/ml) which was 3.2 and 5.6 respectively, was neutralized by 0.01M NaOH. Since above the mentioned compounds are only partially soluble in water, the solutions were centrifuged at 3.000 g for 10 minutes and only the supernatant was added to the incubation Medium 199 at pH 8.2.

- Animals: Mature ewes which have not been pregnant were uses (5.5-6.5 months old, 13.5-16.5kg).

Incubation of hypophysis fragments

hypophysis were harvested immediately after the sacrifice and transported to the laboratory in culture medium. The gland was cut into fragments of 1 mm^3 with a razor blade. About 20 mg of tissue were spread on each stainless grids and incubated in Medium 1991 ml/ grid in 35 mm culture dishes at 37°C and under 95% O_2, 5% CO_2 atmosphere. In this experiment, the samples were first incubated in a medium with none of the compounds for 30 minutes (preincubation) to allow the discharge of lactotropic cells, and to determine the basal level of PRL secretion in each dish. The medium was collected and a fresh medium was then added without (control) or with the above-mentioned compounds at various concentrations and incubation was pursued for one additional hour (first incubation). After collecting the media, the samples were incubated for one additional hour in conditions similar to the first incubation (second incubation). The medium was then collected and kept frozen at 20°C until prolactin measurements. Sterile condition was not necessary in this work since incubation time was short. Nevertheless, all the culture steps were carried out in a culture room under O_2 flux to minimize contamination.

Prolactin measurement

PRL was measured in the culture medium using a radioimmunoassay (RIA) in duplicate samples. In all cases, the hormone concentration was measured in incubation media of each dish after the pre-, the first and the second incubation. The hormonal content of the preincubation medium reflects the basal secretion level of each hypophysis, and it depends on the amount and the quality of the tissue present in each dish. The PRL content of the first and second incubation media reflects of the added compounds. In each experiment, ten dishes were used as control and ten dishes with added compounds for each assay. Each result is the mean $\pm$ SD of ten dishes. As expected, the concentration of PRL in the first and second incubation media of the control dishes was lower than in the preincubation medium. This drop in the second incubation was slightly more intensive. Therefor, the PRL level in the first and second incubation media of control dishes was taken as a

reference (100% secretion) and PRL levels in each group of dishes were compared to it.

Results

Effect of pectic acid on PRL secretion

Fragments of hypophysis were incubated for the sequential in culture media with various concentrations of pectic acid (25-400 µg/ml). Preliminary experiments have shown that some pectic derivatives such as pectic acid are capable of stimulating PRL release (Sepehri *et al.*, 1990). The present results confirm this fact. The maximum stimulation was in the range of 50-150 µg/ml concentrations. Pectic acid was less stimulatory at concentrations higher than 250 µg/ml. These results were observed in the first and second incubations in a similar way (Figs. 1 & 2). Figure 1 shows that various concentrations of pectic acid stimulate PRL secretion in a significant manners ($p<0.05$) at the concentration of 50 and 100 µg/ml. In the dishes containing variable amounts of lactotropic cells, PRL secretion level was different. It was therefore difficult to compare the PRL levels, directly. For this reason, the results are expressed as the percentage, of stimulation with respect to the control (Fig. 2). Figure 2, shows that pectic acid stimulated PRL secretion (from 125% to 178%).

Effect of ?ª-glucan on PRL Secretion

Various concentrations of -glucan (25-500 µg/ml) added to the incubation media of hypophysis fragments showed a clear effect on PRL secretion (Figs. 3 & 4). The maximum stimulation was in the 200-400 µg/ml range of concentration. At concentrations more than 400 µg/ml, -glucan had less stimulatory effect. These results were observed in the first and second incubations in a similar way. Results in Figure 3 shows the stimulatory effect of -glucan on PRL secretion, which is significant ($P<0.05$ or $P<0.01$) at concentrations of 300 to 350 µg/ml. Figure 4 which shows the percentage of PRL secretion in various concentrations of -glucan, indicates that PRL secretion was strongly stimulated (from 103% to 230%).

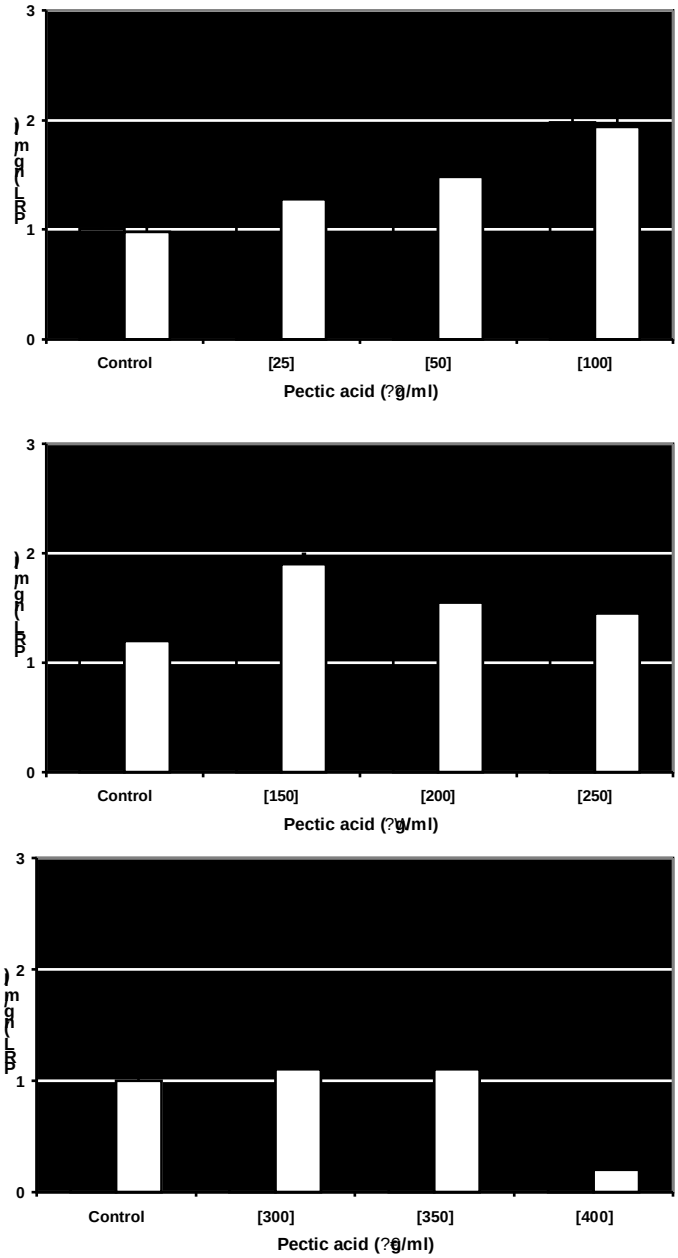

Figure 1 – Effect of various concentrations of petctic acid on PRL secretion from ewe pituity fragments in the first ?œand second ?œ incubations. Each result is the mean ?SEM of ten dishes. ?p<0.05

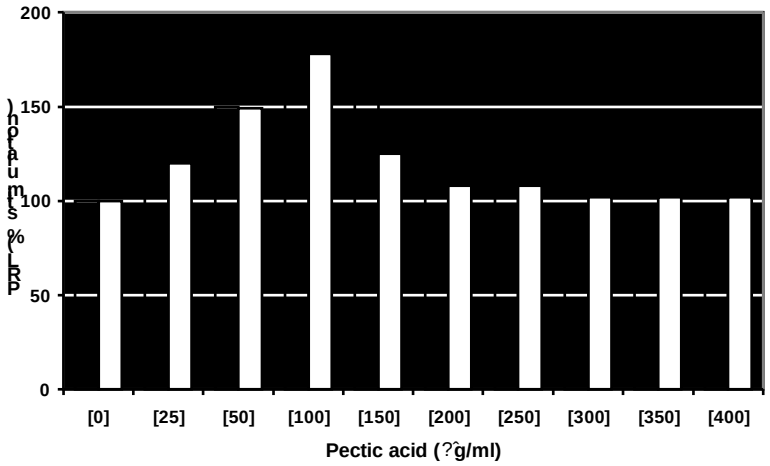

Figure 2 – Effect of pectic acid on PRL secretion in the first ?ó **and second** ?“ **incubations. Results are expressed in percentage in respect to controls.**

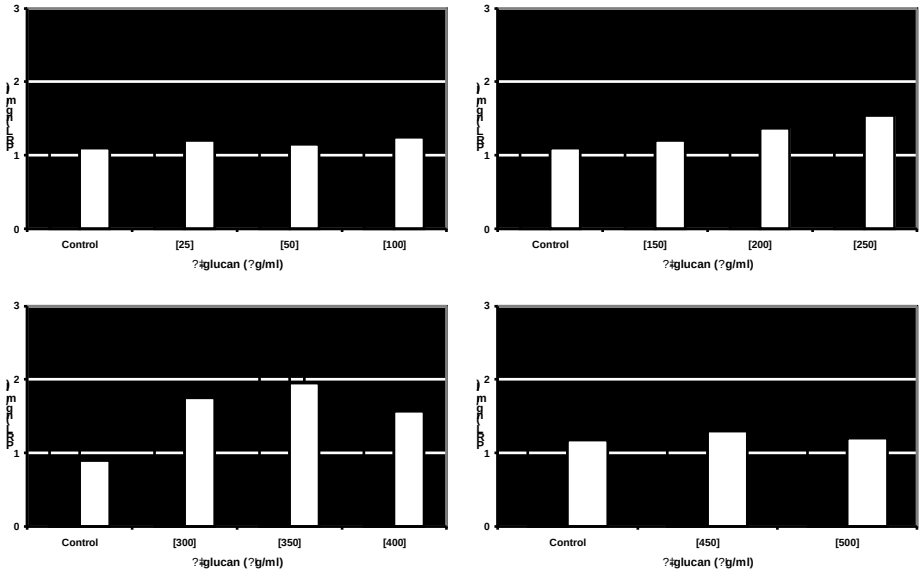

Figure 3 – Effect of various concentrations of ?ŧglucan on PRL secretion from ewe pituitary fragments in the first ?k **and second** ?k **incubations. Each result is the mean ?ŧSEM of ten dishes. ?ß<0.05; ??p<0.01**

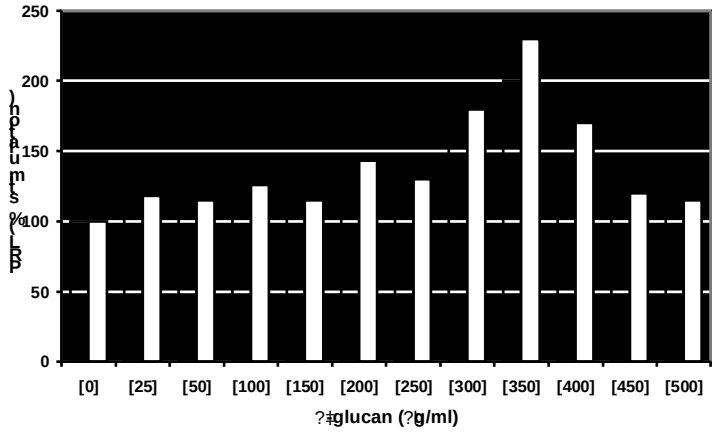

Figure 4 – Effect of ?Ø glucan on PRL secretion in the first ?Ø and second ?x incubations. Results are expressed in percentage in respect to controls.

Conclusion

The mechanism of action of lactogenic plant extracts on PRL secretion is unknown. Pectic substances and -glucan were shown to stimulate, *in vivo*, PRL, GH and possibly cortisol secretion, to induce -casein synthesis in mammary gland. The data reported here clearly show that these compounds stimulate PRL secretion when added to ewe hypophysis fragments. It was therefore concluded from these data that these compounds act as unspecific secretagogues through a direct effect on PRL secretion on lactotropic cells of pituitary. Previous work carried out *in vivo* has shown that GH secretion was also stimulated by these compounds, whereas it was never stimulated *in vitro*. One possible explanation is that GH releasing factor (GHRH) rather than GH secretion is directly affected by the plant extracts *in vivo* (Sepehri,1991). Alternatively, GH secretion may be too low *in vitro* to be significantly stimulated by the added compounds. Pectic substances and -glucan exhibit rather limited homologies of structure and it seems that they act through different mechanisms. However, one point remains striking. Both polymeric compounds studied here are direct precursors of substances named elicitors which recognize specific

receptors on plant cells, act as vegetal hormones and induce the expression of certain genes and defensive responses (Ryan, 1987). No receptor has been identified in the cells of higher vertebrates for these compounds. A possible hypothesis is that pectic substances and -glucan have some structural homologies with extracellular matrix of mammalian cells and the active compounds of plant extracts might affect cellular secretion by binding to these receptors. It remains of find which cellular receptors are involved in which part of cell and by which mechanism of action the stimulatory effects of active compounds of plant extracts on PRL secretion are mediated

Acknowledgments

The Tehran University which provided financial support of this study is fully acknowledged. The authors wish to thank Dr. K. Bahar who provided facilities to perform radioimmunoassays.

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