β-Glucan and Pectin Derivatives Stimulate Prolactin Secretion from Hypophysis *In Vitro*

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Abstract. **Previous work has shown that various plant extracts administered to animals stimulate milk protein synthesis through the secretion of prolactin. It has also been shown that β-glucan and pectin are the active molecules capable of stimulating prolactin release *in vivo* after intravenous injections. In this work, it is shown that β-glucan and several pectin derivatives are able to stimulate prolactin secretion from hypophysis fragments incubated for 2 hr in a synthetic medium.** [P.S.E.B.M. 1990, Vol 194]

Plant extracts are widely used throughout the world to stimulate various physiologic functions. It is generally admitted that beer, gossipium, euphorbia extracts, and so forth have the capacity to enhance milk secretion in lactating woman. In a recent work, Sawadogo *et al.* (1–3) have shown that plant extracts and beer induced β-casein secretion in virgin rats when administered through the oral route. It was observed that the activity of the plant extracts is correlated with their capacity to stimulate prolactin secretion (3–5). On the other hand, the extracts proved to stimulate secretion of growth hormone (GH) and cortisol in addition to prolactin when injected intravenously (4, 5). A partial purification of the active compounds revealed that they are rich in pectin in most cases (4) and rich in β-glucan in beer, malt, and barley extracts (2). Pure pectins and some of their derivatives (3) and pure β-glucan (2, 6) showed a strong capacity to trigger prolactin and GH secretion. From these data, it was concluded that pectic substances and β-glucan are the active lactogenic substances present in plants and that their effects are mediated through the secretion of prolactin and possibly of GH and cortisol. In a preliminary study, it was observed that pectic acid is able to induce the secretion of prolactin, GH, and luteinizing hormone from rat hypophysis kept under perifusion (7). Moreover, pectic substances were shown to stimulate casein secretion from incubated mammary fragments explanted from lactating rabbits (3).

It was therefore concluded that the lactogenic substances act as unspecific secretagogues rather than as specific stimulators of the hypothalamus-hypophysis axis. The present work was undertaken to determine whether pectin substances and β-glucan stimulate prolactin secretion *in vivo* through a direct action on pituitary cells. For that purpose, fragments of hypophysis were incubated with the lactogenic substances and prolactin release was measured in the incubation medium before and after the addition of pectins and β-glucan.

Materials and Methods

Chemicals. Pectic acid was from Fluka, glucuronic acid and dextran from Serva, and monomeric D-galacturonic acid, polygalacturonic acid, and β-glucan from Sigma. Sodium monomeric D-galacturonate was purified from sugar beet pectin after an hydrolysis following the pilot plant process of Sucre Union France Co. Partially digested polygalacturonic acid (PGA) was obtained as follows. PGA (25 g) was dissolved in 0.1 *M* sodium bicarbonate (1 liter). The pH of the solution dropped from 8.2 to 4.8 and 1 ml (208 units) of pectinolytic enzyme solution (Pektolase-LX Grindsted) was added. After stirring for 1 hr at 30°C, the reaction was stopped by heating to 80°C. After cooling, the solution was clarified by filtration through Celite. Oligogalacturonic acids were precipitated by an addition of pure ethanol and dried. For identification of oligogalacturonic acids, the high-performance anion exchange chromatography was used. This partially hydro-

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lyzed PGA was composed of 6% galacturonic acid monomer and oligomers ranging from 2 to 16 mers.

In all cases, the solutions of pectin derivatives were neutralized by an addition of NaOH before being added to the incubation medium. β-Glucan from Sigma was only partially soluble in water. The fraction was centrifuged at 3000*g* for 10 min and only the supernatant was added to the incubation medium. Results refer to the total amount of β-glucan including the discarded insoluble part.

Incubation of Hypophysis. Hypophysis from mature nonpregnant and nonlactating ewes were collected in slaughterhouses. Hypophysis was cut into fragments of 1 mm^3 with a razor blade. About 20 mg of tissue were spread on grids and they were incubated for 1 hr in Medium 199 (1 ml/grid in 35-mm culture dishes) under 95% O_2-5% CO_2 atmosphere. After 1 hr, the medium was collected and a fresh medium was added with or without the above-mentioned compounds. The incubation was pursued for 1 additional hr. The medium was then collected and kept frozen until measurements of hormones.

Measurements of Prolactin and GH. Radioimmunoassays already described were used to evaluate the hormones in the culture medium (4). In all cases, hormone concentration was evaluated in the incubation medium of each dish after the first and second incubation. The hormonal content of the first incubation medium reflects the basal secretion level for each hypophysis and the amount of tissue present in each dish. The hormonal content of the second incubation medium reflects the effect of the added compounds. In each experiment, five dishes were used as a control. As expected, the concentration of prolactin in the second incubation medium of the control dishes was lower than in the first (Fig. 1). Assuming that the drop of hormone secretion was proportionally similar in all dishes, the hormone level in the second incubation medium of the control dishes was taken as the reference (100% secretion). In this way, each group of dishes was compared at best to itself. Five dishes with added compounds were used for each determination. Direct measurements giving prolactin concentration as those in Figure 1 are the mean ± SE of five dishes from the same animal. Other results (each point being the mean of five determinations) are expressed as the percentage of secretion referred to the 100% obtained in the absence of the stimulators. Data shown in Figures 2–5 summarize results obtained from independent incubations using different animals.

Results

Effect of Pectic Compounds on Prolactin Secretion. Fragments of hypophysis were preincubated for 1 hr in the culture medium and then in a similar medium with or without pectic acid or β-glucan. Results in Figure 1 clearly show that both pectic acid and β-glucan independently stimulated very significantly prolactin secretion. The two compounds were tested with different animals. The data in Figure 1 indicate that the basal level of prolactin secretion is a function of each animal. On the other hand, in the dishes containing variable amounts of tissue, their content in secreted prolactin was different. It was therefore difficult to compare directly the prolactin levels. For these reasons, the results in the following experiments were expressed as the percentage of stimulation above the control in each group of dish as explained in Materials and Methods.

Several pectic compounds, pectic acid, polygalacturonic acid, and partially digested polygalacturonic acid were all capable of stimulating prolactin secretion (Fig. 2). The maximum stimulation was in the 25–200 μg/ml range of concentration. At 400 μg/ml the pectic compounds were less stimulatory. This may be due to some toxic effect.

Pectins are composed essentially of linear 1–4 α-linked D-galacturonic acid chain. In order to evaluate whether the active structure in pectin is the monomeric galacturonic acid, this compound was added to the incubation medium. Results shown in Figure 2 indicate

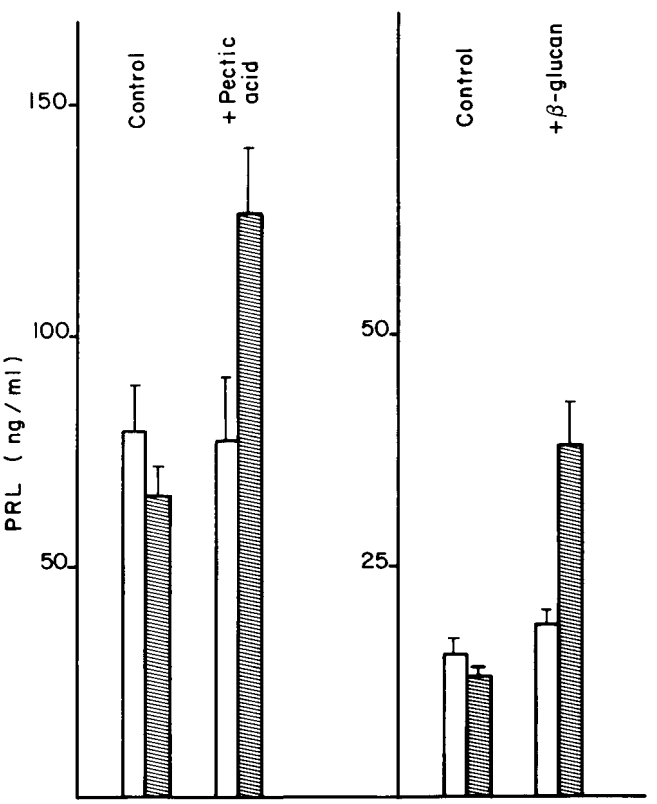

Figure 1. Effect of pectic acid and β-glucan on the secretion of prolactin. Fragments of hypophysis were incubated for 1 hr in Medium 199 (□) and in the new Medium 199 with (+) or without (−) pectic acid (100 μg/ml) or β-glucan (100 μg/ml) (▨). The control contained medium only during the second incubation. Each result is the mean ± SE of five dishes.

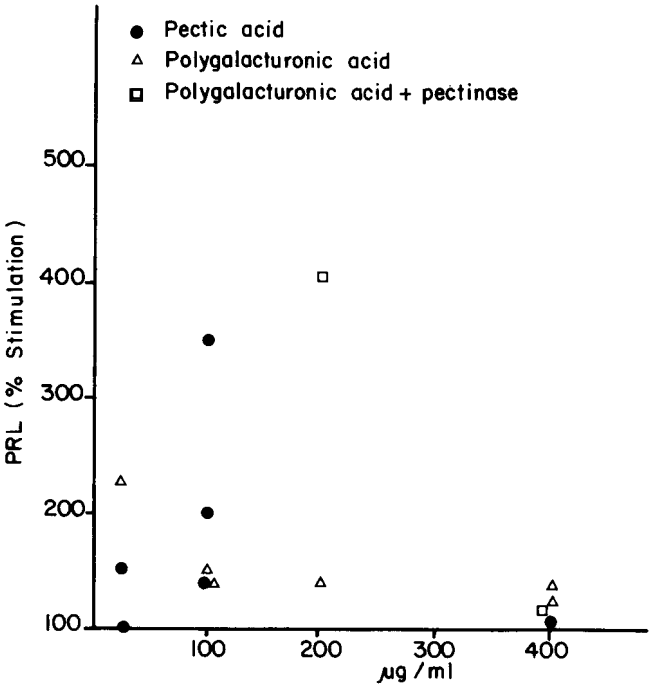

Figure 2. Effect of various pectic compounds on prolactin secretion. Pectic acid, polygalacturonic acid, and partially digested polygalacturonic acid were added at various concentrations during the second phase of incubation. Results are expressed as the percentage of the control in the absence of stimulators.

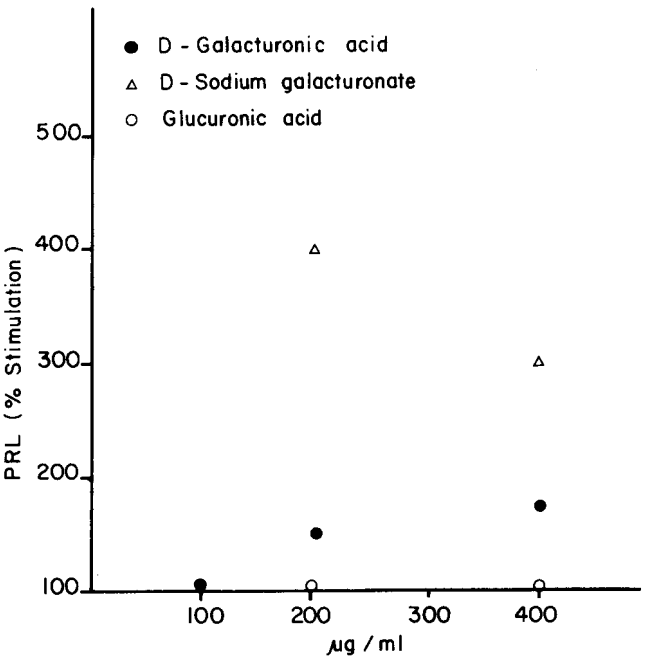

Figure 3. Effect of monomeric D-galacturonic acid and glucuronic acid on prolactin secretion. The compound was added during the second incubation as described in Materials and Methods and in the legends to Figures 1 and 2. Results which are the mean of five independent determinations are expressed as the percentage of stimulation of prolactin secretion.

that monogalacturonic acid had some capacity to trigger prolactin secretion.

As a matter of control, glucuronic acid, which is a carbohydrate somewhat similar to galacturonic acid, was assayed in the same test. This acidic saccharide remained ineffective at any concentration (Fig. 3). This fact was also previously observed *in vivo* (4).

Effect of β-Glucan on Prolactin Secretion. Various concentrations of β-glucan added to the incubation medium of the hypophysis fragments showed a clear effect on prolactin secretion (Fig. 4). Another neutral polysaccharide, dextran, was inactive, at least at the concentration of 100 µg/ml.

Effect of Pectic Substances and β-Glucan on GH Secretion. Our previous work carried out mainly *in vivo* has shown that GH secretion is also stimulated by pectic substances and β-glucan (2–7), although somewhat differently. A comparison of the *in vivo* stimulation of GH and prolactin secretion indicated that a stimulation of GH secretion never or very rarely occurred when prolactin secretion was not stimulated. On the contrary, a stimulation of prolactin secretion was not in all cases accompanied by a parallel stimulation of GH secretion. This suggested that the GH secretion is less sensitive to the plant extracts or that GH secretion was sensitive in a narrower range of concentration of plant extracts than prolactin secretion. The *in vitro* test shown in Figure 5 revealed that GH secretion was insensitive to pectic compounds and to β-glucan in these experimental conditions.

Conclusion

The data reported here clearly show that both pectic substances and β-glucan stimulate prolactin secretion when added to fragments of ewe hypophysis. To the best of our knowledge, this fact is reported for the first time. These results are in good agreement with those previously obtained *in vivo* in the ewe (2–6), rat (3), and rabbit (unpublished results). These compounds markedly stimulate prolactin secretion when administered intravenously. Partially hydrolyzed polygalacturonic acid was active both *in vivo* (3) and *in vitro* (Fig. 2). Unexpectedly, the monomeric D-galacturonic acid also exhibited some activity *in vitro* whereas repeated experiments revealed that it was completely inactive *in vivo* (4 and unpublished data). The discrepancy between these data is not easy to understand. One possible explanation is that D-galacturonic acid in the monomeric form is rapidly eliminated from blood circulation when injected intravenously and is poorly available for hypophysis. A preliminary experiment showed that the short oligogalacturonic acid polymers are not active *in vivo* (3). Experiments are in progress to determine the minimum size of oligogalacturonic acid which is active *in vivo* and to determine whether other carbohydrates present in pectin in addition to galacturonic acid are required.

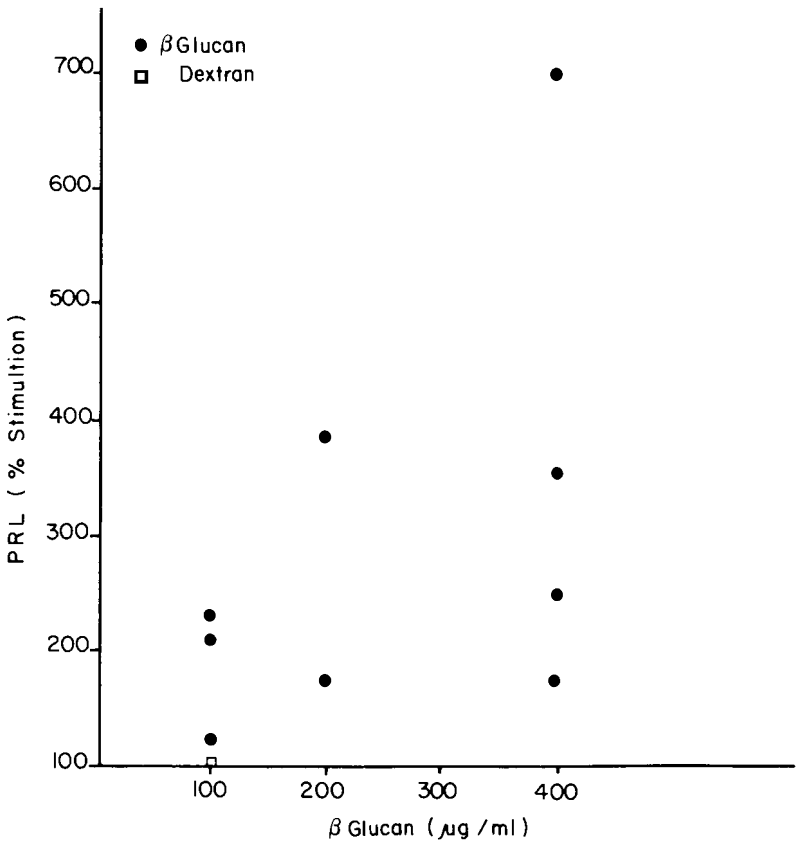

Figure 4. Effect of β-glucan on prolactin secretion. The soluble fraction of β-glucan was added to the second incubation medium at various concentrations. Prolactin secretion was expressed as the percentage of stimulation as in described in the legends to previous figures.

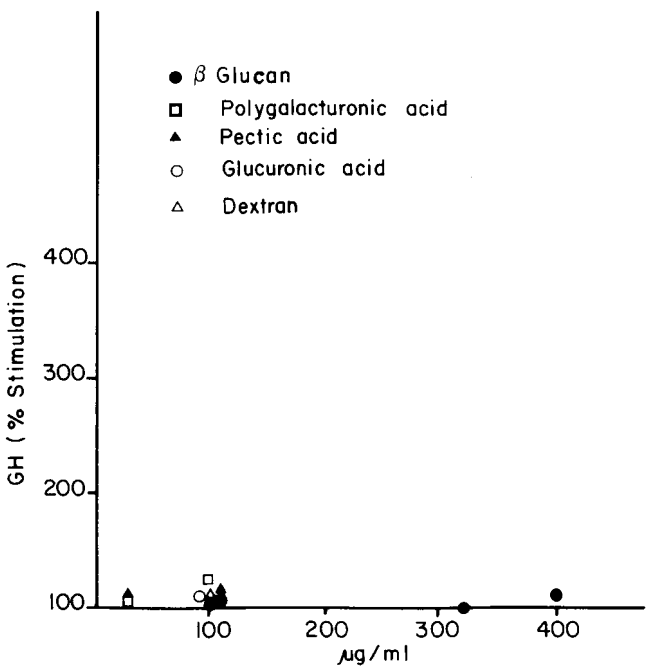

Figure 5. Effect of pectic substances and β-glucan on GH secretion. Some of the medium already tested in prolactin concentrations (Figs. 1–5) were assayed for their content in GH. Results are the mean of five independent determinations.

The mechanism of action of pectic substances and β-glucan on prolactin secretion is unknown. *In vivo* and *in vitro* experiments argue in favor of the idea that they act as rather unspecific secretagogues since they stimulate the secretion of several unrelated hormones and even of casein (3, 4, 7). The data reported here confirm that prolactin secretion is more readily stimulated than GH secretion. It is not known why GH secretion was never stimulated *in vitro*. It is conceivable that growth hormone-releasing factor rather than GH secretion is directly affected by the plant extracts *in vivo*. If so, a negative result *in vitro* is not surprising. Moreover, the pectin substances and β-glucan therefore showed some specificity of action *in vitro* since glucuronic acid remained inactive and GH secretion was unaffected.

Pectic substances and β-glucan exhibit rather limited analogies of structure and it is not easily conceivable that they act through the same cellular mechanism. One point remains striking: both polymeric compounds studied here are the direct precursors of substances named elicitors (8). Elicitors are fragments of pectins and β-glucan which recognize specific receptors on plant cells and act as vegetal hormones. To the best of our knowledge, no receptor has been so far identified in cells of higher vertebrates for pectic substances or β-

glucan. An interesting hypothesis is that the structure of pectic substances and β-glucan share some structural analogies with extracellular matrix of mammalian cells. The plant extracts might affect secretion in these cells by recognizing the cellular receptors for extracellular matrix components.

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