Differential Effects of Glycosylated and Nonglycosylated Prolactin on Islet Cell Division and Insulin Secretion (43582)

Y. N. SINHA*,[1] AND R. L. SORENSON[†]

Whittier Institute for Diabetes and Endocrinology, Scripps Memorial Hospital, La Jolla, California 92037 and Department of Cell Biology and Neuroanatomy,[†] University of Minnesota, Minneapolis, Minnesota 55455*

Abstract. **A growing body of evidence suggests that prolactin (PRL) is a potent regulator of the structure and function of the islets of Langerhans, but PRL is a polymorphic hormone that exists in several molecular forms. Therefore, it is important to know whether glycosylated PRL, a major structural variant of the hormone in several species, has an effect different from that of the nonglycosylated PRL on islet function. This *in vitro* study examined the differential effects of glycosylated and nonglycosylated porcine PRL on cell division and insulin secretion from neonatal rat islets, and compared these results with those produced by homologous rat PRL. The nonglycosylated porcine PRL produced modest stimulation of cell division and insulin secretion from rat islets, but glycosylated porcine PRL had no significant effects. The stimulations produced by nonglycosylated porcine PRL were much weaker in comparison to those produced by the homologous rat PRL. The results show differential effects of the two structural variants of porcine PRL on rat islet function. Although these findings must be confirmed in a homologous system, the results present the possibility that the structural form of the PRL molecule available to the islet tissue may be crucial for its normal functioning.**

[P.S.E.B.M. 1993, Vol 203]

Considerable evidence has been accumulated demonstrating that prolactin is a potent regulator of the structure and function of islets of Langerhans (1–8). Two of the most important of these features is prolactin's ability to induce islet B cell division and enhance insulin secretion. In addition to prolactin's direct effects on islets, it also may alter insulin metabolism by inducing a state of peripheral insulin resistance (1, 9), similar to that observed during pregnancy (10).

Recent studies have revealed the existence of prolactin (PRL) in several molecular forms (11). Among the different forms so far identified, glycosylated PRL (G-PRL) is a major constituent, forming as much as 40% of the total pituitary PRL in some species (12). Moreover the ratio of the nonglycosylated to glycosylated forms of PRL are subject to change, as has been demonstrated during pregnancy (13).

In this report, we examined the direct effects of glycosylated and nonglycosylated prolactins on insulin secretion and islet cell division in neonatal rat islets *in vitro*. Since rat pituitaries are small and offer very little glycosylated PRL for isolation, hormones purified from porcine pituitaries were used for these studies.

Materials and Methods

Islet Isolation and Culture. Neonatal rat islets were isolated from 3- to 5-day-old rats pooled from two or more litters (Sprague-Dawley; Holtzman, Madison, WI) by a nonenzymatic culture method described previously (14). Groups of 30 islets were transferred to 24-well plates (Costar, Cambridge, MA) and cultured free floating in 2 ml of RPMI 1640 medium containing 10 m*M* glucose (Gibco, Grand Island, NY) supplemented with 10% horse serum, 25 m*M* HEPES, and 1% penicillin-streptomycin-fungizone antibiotic-antimycotic.

Determination of Hormone Effect on Insulin Secretion. For each experiment, the multiwells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2 (7). The hormones were added to the

[1] To whom requests for reprints should be addressed at The Whittier Institute for Diabetes and Endocrinology, 9894 Genesee Avenue, La Jolla, CA 92037.

Received May 26, 1992. [P.S.E.B.M. 1993, Vol 203]
Accepted January 21, 1993.

0037-9727/93/2031-0123$3.00/0

cultures as concentrated sterile aqueous solutions. The cultures were carried out for 8 days. The culture medium was replaced with fresh medium every 24 hr and stored frozen for subsequent assay. Insulin concentrations were determined by radioimmunoassay (15) using rat insulin standards (Novo, Danbury, CT).

Bromodeoxyuridine Immunocytochemistry. To estimate islet B cell proliferation, bromodeoxyuridine (BrdU) was added to the culture medium to a final concentration of 10 μM for the final 24 hr of culture. When the cultures were discontinued, the islets were washed, fixed, and immunostained with mouse monoclonal anti-BrdU antibody (Becton-Dickinson, Mountain View, CA). For a secondary antibody, fluoroscein isothiocyanate-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) was used. To determine islet B cell proliferation, the number of BrdU-labeled nuclei/islet was determined by direct observation with conventional epifluorescence microscopy. Slides were coded so that the evaluator was unaware of the treatment groups. Details of this procedure, for islet studies, have been reported previously (8). At least 100 islets were examined from each experimental group.

Hormone Preparations. The rat PRL, obtained from the National Hormone and Pituitary Program, (NIDDK-rPRL-B-6, 25.0 IU/mg) had 0.35% growth hormone contamination by weight. Porcine PRL (USDA pPRL B-1) was obtained from the U.S. Department of Agriculture Animal Hormone Program. It contains both glycosylated and nonglycosylated forms in an approximately 40:60 ratio. Nonglycosylated porcine PRL and glycosylated porcine PRL were purified in our laboratory as described previously (16). The glycosylated form used consisted of two types—one that binds to concanavalin A (retained fraction; G^4 of Ref. 16) and one that does not bind to concanavalin A (unretained fraction; G^2 of Ref. 16). Due to the limited availability of some of the hormones, a single dose of 500 ng/ml was used for these experiments. We have shown previously that 500 ng/ml of PRL has a maximum effect on islets (7, 8). The stimulatory effect of PRL on islet cell function is highly specific, as the action is completely blocked by PRL antibodies (Table I).

Data Analysis and Presentation of Results. For an individual experiment, each experimental group was replicated six times and each experiment was repeated twice. All results are expressed as the mean ± SE of n observations. Statistical differences between means were assessed by analysis of variance with Bonferroni's post-hoc test for multiple comparisons.

Results

Effect of PRL Variants on Islet Cell Proliferation. To compare the effects of rat PRL (rPRL), porcine PRL (pPRL), nonglycosylated pPRL, and two forms of glycosylated pPRL on islet cell division, the data were plotted in Figure 1. In control islets, the mean number of BrdU-labeled nuclei/islet was 1.2 ± 0.16. rPRL treatment resulted in an 8-fold increase in BrdU-labeled nuclei/islet (9.5 ± 0.6; $P < 0.001$). pPRL treatment resulted in a 3-fold increase in BrdU-labeled nuclei/islet (3.4 ± 0.4; $P < 0.006$). Similarly, the nonglycosylated form of pPRL increased BrdU labeling 3-fold (3.1 ± 0.3; $P < 0.01$), whereas the glycosylated forms of pPRL were ineffective (2.0 ± 0.2 and 1.9 ± 0.2; $P > 0.10$). Results from two separate experiments were very similar.

Table I. Blockage of PRL Action on Islet Cell Function by Antiserum to PRL

Group	Insulin secretion (mU)	BrdU-labeled nuclei/islet
Control	15 ± 2*	2.5 ± 0.3*
Ovine PRL (500 ng/ml)	24 ± 2†	35.0 ± 5.0†
Ovine PRL (500 ng/ml) + anti-ovine PRL serum (50 μl)	7 ± 1‡	1.5 ± 0.5*

Note: Means with common symbols (*, †, ‡) are statistically not different from one another ($P > 0.05$).

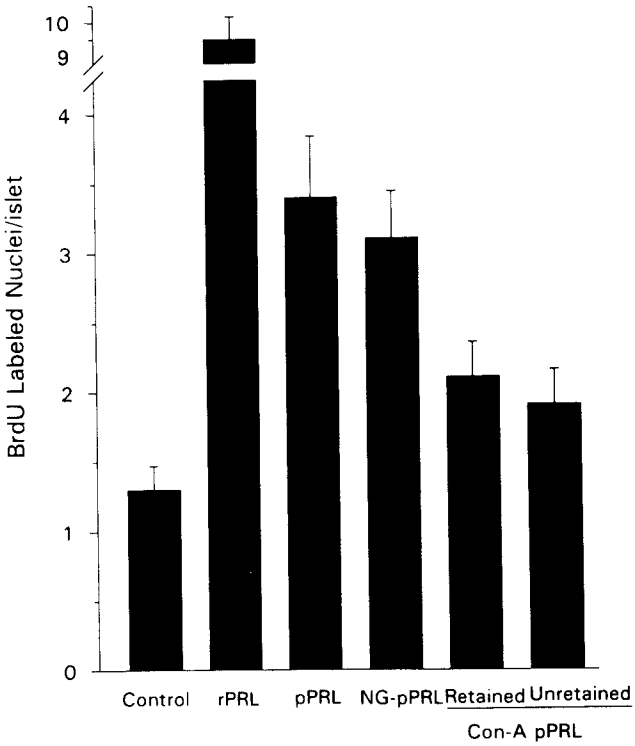

Figure 1. Effect of nonglycosylated and glycosylated prolactins on BrdU incorporation into neonatal islets. Preparations predominantly containing nonglycosylated prolactin, such as rPRL, pPRL, and nonglycosylated pPRL (NG-pPRL), significantly enhanced BrdU incorporation ($P < 0.01$, $n = 6$). Neither variant of the glycosylated prolactin, concanavalin A (ConA)-retained or unretained, affected BrdU incorporation ($P > 0.10$, $n = 6$).

Effect of PRL Variants on Insulin Secretion. To compare the effects of rPRL, pPRL, nonglycosylated pPRL, and two forms of glycosylated pPRL on insulin secretion, the islets were cultured in the presence of 500 ng/ml of each of the hormones for 8 days. Culture media were changed daily and the amount of insulin secreted was determined. The cumulative insulin secreted during the 8-day culture period for the control group was 6.3 ± 0.6 mU (Fig. 2). rPRL treatment resulted in a 2-fold increase in insulin secretion (12.1 ± 0.4 mU; $P < 0.001$). pPRL (8.2 ± 0.4 mU) and nonglycosylated pPRL (8.5 ± 0.6 mU) significantly ($P < 0.05$) enhanced insulin secretion, whereas the glycosylated forms of pPRL were ineffective (7.6 ± 0.6 and 8.0 ± 0.4 mU; $P > 0.10$). Results from two separate experiments were very similar.

Discussion

The results of this study demonstrate that porcine PRL is less effective than the homologous rat PRL in stimulating cell division and insulin secretion from rat islets. This is in accord with previous findings comparing human and rat growth hormone (7), and is most likely due to the structural differences between hormones of the two species. Porcine PRL has only 64% sequence homology with rat PRL (17).

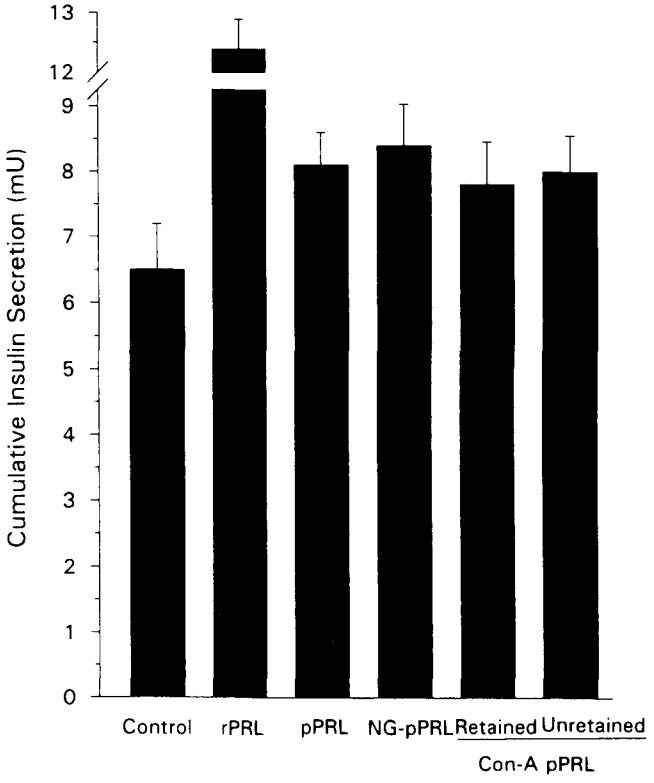

Figure 2. Effect of nonglycosylated and glycosylated prolactins on insulin secretion from neonatal islets. Preparations containing predominantly nonglycosylated prolactin, such as rPRL, pPRL, and nonglycosylated pPRL (NG-pPRL), significantly enhanced insulin secretion ($P < 0.03$, $n = 6$). Neither variant of the glycosylated prolactin, concanavalin A (ConA)-retained or unretained, affected insulin secretion ($P > 0.10$, $n = 6$).

It is also obvious from these results that the nonglycosylated form of porcine PRL is more effective than the two glycosylated forms tested in stimulating cell proliferation and insulin secretion from rat islets. Markoff *et al.* (18) found the ovine G-PRL also to have no effect on insulin release from rat islets over a 6-day culture period. PRL binding sites have been detected in islets of Langerhans (19, 20). In addition, immunoreactive PRL has been localized within the cytoplasm of the insulin-secreting B cells (21). Our studies suggest that the PRL which binds to islets and which can be detected within B cells is likely to be the nonglycosylated form of the hormone. The structure of the carbohydrate in porcine G-PRL is not known, but it appears that the attachment of the sugar moiety to the molecule alters it sufficiently such that it no longer is able to act on the islet tissue. Thus, glycosylation seems to confer tissue specificity to the molecule for the expression of biologic action.

The clinical implications of the inability of G-PRL to stimulate B cell function could be significant. B cell function could become adversely affected in individuals who secrete predominantly G-PRL or inadequate amounts of nonglycosylated PRL. Many studies indicate that PRL is responsible for the maintenance of immunocompetence in animals and human beings (22). Thus, a preponderance of G-PRL, a less bioactive form of the hormone, could play an etiologic role by way of the disturbed immune function as well in diabetic patients.

These results document the differential effects of PRL variants on B cell division and function, and point to the importance of giving due consideration to the structural heterogeneity of PRL in studies of its biologic and pathologic actions.

This work was supported by NIH Grants DK-32311 (to Y. N. S.) and DK-33655 (to R. L. S.). The authors are grateful to the hormone distribution program of the NIH for the gift of rat and porcine PRL used in this study.

1. Landgraf R, Landgraf-Leurs MMC, Weissmann A, Horl R, vonWerder K, Scriba PC. Prolactin: A diabetogenic hormone. Diabetologia **13**:99–104, 1977.
2. Gustafson AB, Banasiak MK, Kalkhoff RK, Hagen RC, Kim JH. Correlation of hyperprolactinemia with altered plasma insulin and glucagon: Similarity to effects of late human pregnancy. J Clin Endocrinol Metab **51**:242–246, 1980.
3. Nielsen JH. Effects of growth hormone, prolactin, and placental lactogen on insulin content and release, and deoxyribonucleic acid synthesis in cultured pancreatic islets. Endocrinology **110**:600–606, 1982.
4. Sorenson RL, Parsons JA. Insulin secretion in mammosomatotropic tumor bearing and pregnant rats: A role for lactogens. Diabetes **34**:337–341, 1985.

5. Sorenson RL, Johnson MG, Parsons JA, Sheridan JD. Decreased glucose stimulation threshold, enhanced insulin secretion, and increased beta cell coupling in islets of prolactin-treated rats. Pancreas **2**:283–288, 1987.
6. Sorenson RL, Brelje TC, Hegre OD, Marshall S, Anaya P, Sheridan JD. Prolactin (*in vitro*) decreases glucose stimulation threshold, enhances insulin secretion, and increases dye coupling among B-cells. Endocrinology **121**:1447–1453, 1987.
7. Brelje TC, Allair P, Hegre O, Sorenson RL. Effect of prolactin *versus* growth hormone on islet function and the importance of using homologous mammosomatotrophic hormones. Endocrinology **125**:2392–2399, 1989.
8. Brelje TC, Sorenson RL. Role of prolactin *versus* growth hormone on islet B-cell proliferation *in vitro*: Implications for pregnancy. Endocrinology **128**:45–47, 1991.
9. Serri O, Beauregard H, Rasio E, Hardy J. Decreased sensitivity to insulin in women with microprolactinomas. Fertil Steril **45**:572–574, 1986.
10. Buchanan TA, Metzger BE, Freinkel N, Bergman RN. Insulin sensitivity and B-cell responsiveness to glucose during late pregnancy in lean and moderately obese women with normal glucose tolerance or mild gestational diabetes. Am J Obstet Gynecol **162**:1008–1014, 1990.
11. Sinha YN. Prolactin variants. Trends Endocrinol Metab **3**:100–106, 1992.
12. Pankow YuA, Butnev VYu. Multiple forms of pituitary prolactin, a glycosylated form of porcine prolactin with enhanced biological activity. Int J Pep Protein Res **28**:113–123, 1986.
13. Markoff E, Lee DW, Hollingsworth DR. Glycosylated and non-glycosylated prolactin in serum during pregnancy. J Clin Endocrinol Metab **67**:519–523, 1988.
14. Hegre O, Marshall S, Schulte BA, Hickey GE, Williams F, Sorenson RL, Serie JR. Non-enzymic *in vitro* isolation of perinatal islets of Langerhans. In Vitro **19**:611–620, 1983.
15. Morgan CR, Lazarow A. Immunoassay of insulin: Two antibody system. Diabetes **12**:115–126, 1963.
16. Sinha YN, DePaolo LV, Haro LS, Singh RNP, Jacobsen BP, Scott KE, Lewis UJ. Isolation and biochemical properties of four forms of glycosylated porcine prolactin. Mol Cell Endocrinol **80**:203–213, 1991.
17. Nicoll CS, Mayer GL, Russell SM. Structural features of prolactins and growth hormones that can be related to their biological properties. Endocr Rev **7**:169–203, 1986.
18. Markoff E, Beattie GM, Hayek A, Lewis UJ. Effects of prolactin and glycosylated prolactin on (Pro) insulin synthesis and insulin release from cultured rat pancreatic islets. Pancreas **5**:99–103, 1990.
19. Tesone M, Oliveira-Filho RM, Charreau EH. Prolactin binding in rat Langerhans islets. J Recept Res **1**:355–372, 1980.
20. Polak M, Scharfmann R, Ban E, Haour F, Postel-Vinay MC, Czernichow P. Demonstration of lactogenic receptors in rat endocrine pancreas by quantitative autoradiography. Diabetes **39**:1045–1049, 1990.
21. Meuris S, Verloes A, Robyn C. Immunochemical localization of prolactin-like immunoreactivity in rat pancreatic islets. Endocrinology **112**:2221–2223, 1983.
22. Berczi I, Nagy E. The effect of prolactin and growth hormone on hemolymphopoietic tissue and immune function. In: Berczi I, Kovacs K, Eds. Hormones and Immunity. Lancaster, UK: MTP Press Ltd., pp145–171, 1987.