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Effect of Glycosylated and Non-glycosylated Prolactin on the Proliferation of Normal Human Lymphocytes

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Summary

We evaluated the effects of $2.5x10^{-10}$ M or $5x10^{-10}$ M concentrations of human pituitary prolactin (pPRL), human recombinant non-glycosylated (NG-PRL) and glycosylated (GL-PRL) prolactin on the proliferation of normal human lymphocytes with or without coactivation by interleukin-2 (IL-2). None of the PRL forms alone affected the lymphocyte proliferation in a serum-free medium, however, the stimulatory activity of IL-2 was significantly potentiated with all 3 PRL variants. Since the $5x10^{-10}$ M concentrations of individual PRLs exerted the same effects, this result suggests that GL-PRL in primary lymphocyte culture is not a less mitogenic form, if sufficient amounts of IL-2 are available.

Key words
Glycosylated prolactin – Non-glycosylated prolactin – Pituitary prolactin – Human lymphocytes

Introduction

The role of PRL in the regulation of immune functions has been well documented: lymphoid cells express surface receptors for PRL (Russel *et al.* 1984) and T-cells produce mRNA for PRL (Pellegrini *et al.* 1992, Jurčovičová *et al.* 1992), which may act by autocrine/paracrine mechanisms. There is also some clinical evidence that hyperprolactinaemia may account for the occurrence of several autoimmune diseases (Walker *et al.* 1993). During T-cell proliferation, PRL cooperates with the main T-cell mitogen IL-2 in a sequence of events: external PRL upregulates surface receptors for IL-2 (Mukherjee *et al.* 1990) which in turn drives the PRL translocation into the nucleus (Clevenger *et al.* 1990). Internalized PRL serves as a growth factor necessary for cells to enter the S phase (Clevenger *et al.* 1992).

PRL in man circulates in glycosylated (GL-PRL) and non-glycosylated (NG-PRL) forms. The GL-PRL form is known to have about 12-fold lower mitogenic activity than NG-PRL as measured by the proliferative effects on the Nb-2 rat thymic lymphoma cell line (Young *et al.* 1990). Since the ratio of GL-PRL and NG-PRL variants, which are not distinguishable by current assays, varies in the peripheral blood in various situations, the biological effects of circulating PRL on lymphoid cells may also be different. Therefore, it was of interest to evaluate the proliferative activities of individual forms of PRL on normal human lymphocytes *in vitro* and to compare them with the effect of pPRL.

Materials and Methods

Human mononuclear cells were isolated on Histopaque-1077 (Sigma) gradients from heparinized venous blood drawn from four male healthy volunteers. The cells were resuspended in a RPMI-1640 medium (Sigma) supplemented with 15 mM HEPES, 2 mM L-glutamine (Gibco BRL), 50 U/ml PEN-STREP (Gibco BRL) and 10 % foetal bovine serum (Gibco BRL) at a concentration of $3x10^6$ cells per ml and stimulated with 5 μg/ml Concanavalin-A (Con-A) (Boehringer) in a humidified atmosphere of 5 % CO_2 and 95 % air for 60 h. The cells were then washed with a serum free RPMI-1640 medium (SFM) containing Gibco Media Supplement-Suspension composed of

insulin 1.0 g^{-1}, sodium selenite 0.67 mg^{-1}, sodium transferrin 0.55 g^{-1}, pyruvate 11 g^{-1} and ethanolamine 0.2 g^{-1} (Gibco BRL Life Technologies), 50 U/ml PEN-STREP, 0.05 % BSA fraction V (Sigma), 2 mM L-glutamine. This was resuspended in the SFM with the addition of methyl-α-p-manopyranoside (0.15 M) to bind the residual Con-A. The cells further expanded with 50 U/ml of IL-2 (Gibco BRL) for 48 h and synchronized in SFM for additional 24 h.

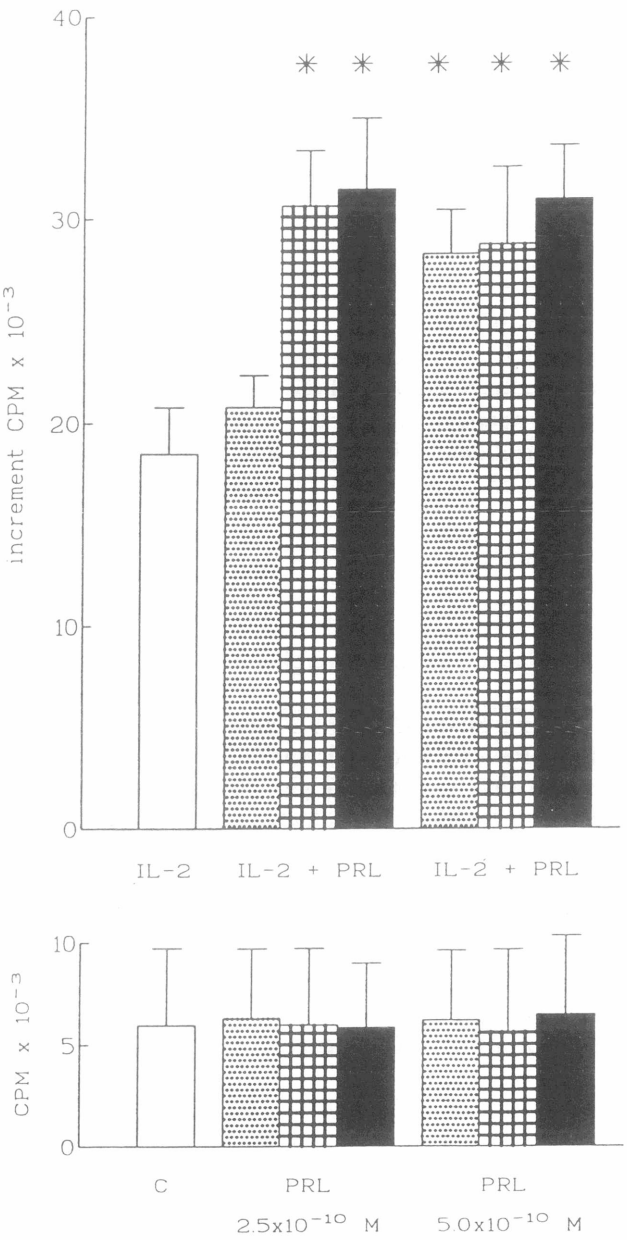

Fig. 1. *Effect of different concentrations of NG-PRL – speckled bars, GL-PRL – checkered bars and pPRL – solid bars on lymphocyte proliferation. Empty bar represents the non-stimulated proliferation (lower part). In the upper part are depicted the differences between the proliferation with and without IL-2. Empty bar represents the increment after IL-2 alone. Results are expressed as means ± S.E.M. (* $p<0.05$ by paired Student's t-test)*

Thereafter, the cells were plated in tri- or quadruplicates in 96 well microtitre plates (10^5 cells per plate in a total volume of 225 μl SFM) and incubated with either human pPRL (NIDDK), or human recombinant NG-PRL, or GL-PRL (Genzyme) at 2.5 x 10^{-10} M or 5 x 10^{-10} M concentrations with or without 50 U/ml IL-2 for 24 h. Control wells were without IL-2 and PRL to assess the background proliferation. To evaluate the effect of IL-2 some wells were incubated without addition of PRL. For the last 8 h the cells were pulsed with 0.5 μCi (18.5 kBq) of ^{3}H-thymidine (NEN Dupont). Then the cells were harvested onto glass filters and the incorporated ^{3}H-thymidine was measured on a β-scintillation counter. The results are expressed as cpm. The statistical significance was calculated using oneway ANOVA and paired Student's t-test.

Results and Discussion

The doses of PRL used (corresponding with 5.7 or 11.5 ng/ml), which represent its normal levels in the circulation, did not affect lymphocyte proliferation alone *in vitro* in a serum-free medium (Fig. 1, lower part). This is in line with the finding that PRL is a necessary but not the only growth factor for lymphocytes to complete the cell cycle (Clevenger *et al.* 1992). Incubation of the cells with IL-2 resulted in pronounced proliferation ($24.5\pm1.5x10^3$ cpm vs. $5.9\pm3.7x10^3$ cpm, $p<0.02$). Since the medium did not contain PRL which is required for IL-2 driven cell growth, this result demonstrates that human T-cells can produce and secrete sufficient amounts of PRL to induce cell proliferation in cooperation with IL-2. The individual forms of exogenous PRL further potentiated the IL-2 effect (ANOVA, $p<0.05$) to the same extent, except for the lower dose of NG-PRL (Fig. 1, upper part). In our experimental design, the GL-PRL did not exhibit less mitogenic activity as has been repeatedly shown in the Nb-2 lymphoma cell bioassay. It should be mentioned that the Nb-2 cell line is derived from cancerous cells which have about 30 times more PRL receptor sites with about 300 times greater affinity than human lymphocytes (Shiu *et al.* 1983, Russell *et al.* 1984). It is therefore not an ideal model for characterizing PRL effects in normal cells. Considering the low number of receptors in lymphocytes, we assume that the binding sites are fairly accessible to both forms of PRL and that after IL-2 stimulation, the GL-PRL can act as an equally active form. These preliminary results suggest that in humans under basal conditions, when the level of circulating PRL is low and supposedly the GL-PRL form is prevailing, endogenous PRL can stimulate the growth of lymphocytes. An important additional factor is IL-2 in sufficient amounts. This may be important in patients with rheumatoid arthritis, where PRL is known to be involved in the progression of the disease. However,

the activity of PRL as measured on Nb-2 cells was shown to be lower than in normal subjects (Berczi *et al.* 1993).

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