***In vitro* studies of the stimulation of insulin secretion and B-cell proliferation by rat placental lactogen-II during pregnancy in rats**

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The present study investigated the effect of rat placental lactogen-II (rPL-II) on insulin secretion and B-cell proliferation of the maternal islets during the last half of pregnancy in rats using a homologous system. Pancreatic islets were isolated from nonpregnant rats and rats at day 13 of pregnancy and cultured for 8 days in medium containing trophoblast culture medium or purified hormones. The medium was changed daily and insulin concentrations were determined by measuring immunoreactivity. The number of proliferating B cells were determined by double staining for both insulin and 5'-bromo-2'-deoxyuridine (BrdU) incorporated into replicating DNA during the last 24 h of incubation. rPL-II-enriched medium, in which trophoblasts of placentae from rats at day 13 of pregnancy were incubated for 8 days, was added to the islet culture system. Insulin concentration in the medium of non-pregnant rat islets was significantly increased and doubled on incubation in 100% trophoblast culture medium. Addition of purified rPL-II to the culture medium of pregnant rat islets stimulated insulin secretion at the concentrations of 50–500 ng ml⁻¹ in a dose-dependent manner. The stimulatory effect of rPL-II on insulin secretion was found to be more than double that with rat prolactin (rPRL) and rat growth hormone (rGH) at 100 ng ml⁻¹. For determination of B-cell proliferation, non-pregnant rat islets were incubated with rPL-II, rPRL or rGH at 1000 ng ml⁻¹ for 8 days and then 10 mmol BrdU l⁻¹ was added during the last 24 h of incubation. rPL-II (5.30 ± 0.36%), rPRL (3.79 ± 0.34%) and rGH (2.87 ± 0.29%) significantly increased the rate of incorporation of BrdU into the B cells compared with that of the control (1.34 ± 0.18%). These results indicate that rPL-II directly regulates insulin secretion and B-cell proliferation of maternal islets during the last half of pregnancy in rats.

Introduction

The glucose demands of the fetus increase markedly with fetal growth, especially during the last half of pregnancy (Metcalf *et al.*, 1994). The maternal pancreatic islets are thought to adapt to this increased demand for glucose by increasing their insulin secretory response and by B-cell proliferation (Green and Taylor, 1972; Parsons *et al.*, 1992). Previous studies have indicated that, in comparison with somatogenic hormones, lactogenic hormones secreted during pregnancy are more potent stimulators for inducing functional and structural changes of pancreatic B cells (Sorenson and Parsons, 1985; Brelje and Sorenson, 1991; Parsons *et al.*, 1992). Although the biological activities of the prolactin-growth hormone families, such as placental lactogen (PL), prolactin (PRL) and growth hormone (GH), on B-cell function have been widely studied (Nielsen 1982; Nielsen *et al.*, 1986; Brelje *et al.*, 1989), much of the work has been carried out with heterologous rather than homologous systems. However, human growth hormone is known to have somatogenic and lactogenic activities when used with rat tissues (Nicoll *et al.*, 1986). Since it is possible that

other members of the prolactin-growth hormone family behave in a similar manner, data obtained from experiments using homologous systems are important for analysing the precise effects of these hormones on their own tissues.

In rodents, two placental lactogens, PL-I, PL-II, have been identified, one is present at mid-pregnancy (PL-I) and the other during the last half of pregnancy (PL-II). PL-I and PL-II are known to possess structural similarity with prolactin rather than GH, and thus, are lactogenic rather than somatogenic hormones in rodents (Nicoll *et al.*, 1986).

Brelje *et al.* (1993) reported that rat PL-I (rPL-I) and mouse PL-I and PL-II (mPL-I, mPL-II) have the potential to enhance islet function by increasing the insulin secretory response and proliferation of B cells in a homologous system. However, no information is available on the role of rPL-II in pancreatic islet function in rats, even though the maximum concentration of rPL-II in maternal circulation in rats is approximately 1000 ng ml⁻¹ (Ogren and Talamantes, 1988; Kishi *et al.*, 1991). Moreover, during the last half of pregnancy, when the glucose demands of the fetus increase markedly, rPL-I disappears from maternal circulation and rPL-II is secreted predominantly as a lactogenic hormone into the maternal circulation.

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The present study was undertaken to investigate the effect of rPL-II on insulin secretion from and B-cell proliferation into the maternal islets during the last half of pregnancy in rats using a homologous system.

Materials and Methods

Animals

Female Sprague-Dawley rats weighing 250–350 g were housed under controlled lighting (12 h light:12 h dark, lights on 08:00 h) and temperature ($23 \pm 1^\circ\text{C}$) conditions with free access to food and water.

Isolation and culture of islets

Pancreatic islets were isolated from adult rats as described by Gotoh *et al.* (1987) with several modifications. Animals were anaesthetized with Nembutal (50 mg kg^{-1}). A polyethylene tube (outer diameter 1 mm) was inserted into the common bile duct, and the distal end of the duct was clamped. Approximately 15 ml of ice-cold Hank's balanced salt solution (HBSS, pH 7.4) with 0.3 mg ml^{-1} collagenase (type XI, Sigma, St Louis, MO) and 2% (w/v) BSA (Sigma) was injected. The distended pancreas was carefully excised to prevent it from bursting and placed into a 50 ml plastic conical tube. After incubation of the distended pancreas for 35 min in a water bath (37°C) without shaking, ice-cold HBSS was added to stop the digestion. The sample was then centrifuged (350 g for 5 min), the supernatant was discarded and 15 ml of ice-cold HBSS was added. After the digested tissue was agitated several times with a pipette, the plastic tube was centrifuged at 160 g for 2 min and the supernatant was discarded. The digested tissue was poured into a 15 ml centrifuge tube containing 3 ml 27% (w/v) Ficoll 400 (Pharmacia Biotech AB, Uppsala) solution and vortexed for 2–3 s. The tissue suspension was overlaid on a discontinuous Ficoll gradient (20.5 and 11%, 4 and 2 ml, respectively) followed by centrifugation at 450 g for 15 min. The islets at the 20.5/11% interface were collected with a Pasteur pipette in RPMI-1640 culture medium (10 mmol glucose l^{-1} , Gibco, Grand Island, NY) which was supplemented by 10 mmol Hepes l^{-1} , 23.8 mmol NaHCO_3 l^{-1} , 5% fetal bovine serum (FBS), 50 mg gentamicin ml^{-1} , 50 U penicillin ml^{-1} , 50 mg streptomycin ml^{-1} , pH 7.4. Groups of 25 islets were transferred to 24-well plates and incubated at 37°C under 95% air:5% CO_2 for up to 8 days. The medium was changed every day and stored at -20°C until assayed. The insulin concentration in the culture medium was assayed using a kit (Shionoria Insulin Kit, Shionogi & Co., Ltd, Osaka).

Culture of trophoblast cells

Culture of trophoblast cells was performed as described by Kishi *et al.* (1993). Briefly, conceptuses were collected from pregnant rats at day 13 of pregnancy. The fetus and decidua basalis were discarded and the basal zone and the labyrinth were collected. The tissue was finely minced and minced tissue was dispersed with dissociation medium containing 0.1% (w/v)

collagenase and 0.002% (w/v) DNase. The cell suspension was centrifuged at 700 g for 5 min, resuspended in 2 ml of dissociation medium and centrifuged at 700 g for 15 min through a 40% (v/v) Percoll density gradient. Cells banded at a density of about 1.049 g ml^{-1} were collected. These cells contained at least three types of trophoblast cells: giant cells with a single nucleus or multinuclei, round cells and small round trophoblast cells. An immunohistochemical study had previously confirmed that approximately 70% of the trophoblast cells obtained by the above procedure are positive for rPL-II (Kishi *et al.*, 1993). The cells obtained from the placentae were resuspended at concentrations of $0.5\text{--}1 \times 10^6$ cells ml^{-1} in SFM-101 medium (10 mmol glucose l^{-1} , insulin free; Nissui, Tokyo) supplemented with 26 mmol NaHCO_3 l^{-1} , 10 mmol Hepes l^{-1} , 50 mg gentamicin ml^{-1} , 50 U penicillin ml^{-1} and 50 mg streptomycin ml^{-1} , pH 7.4. Cells were incubated at 37°C under 95% air:5% CO_2 for up to 6 days. The medium was changed every day and stored as a pool at -20°C until assayed.

Hormones

rPL-II was purified from the placentae of rats at day 19 of pregnancy as described by Kishi *et al.* (1992). The homogeneity of purified rPL-II was monitored with SDS-gel electrophoresis. Purified rPL-II was characterized by a major band of molecular mass of 24.5 kDa and a minor band of a molecular mass of 25.0 kDa under reducing conditions (data not shown). Rat prolactin (NIADDK-rat PRL-B-5) and rat growth hormone (NIADDK-rat GH-B-8) were supplied by NIADDK.

BrdU and insulin immunocytochemistry

The number of proliferating B cells was determined by staining for both insulin and 5'-bromo-2'-deoxyuridine (BrdU) incorporated into the replicating DNA. After 7 days of culture in the presence of the hormone, the islets were incubated with BrdU at a final concentration of 10 mmol l^{-1} for the last 24 h of incubation in the presence of hormone. When the incubation was finished, the islets were washed with HBSS (Mg^{2+} , Ca^{2+} -free; HBSS-F) and digested with 0.1% trypsin (Difco, Detroit, MI), 0.1% (w/v) BSA in HBSS-F for 6–7 min in a shaking water bath at 37°C . Cold HBSS-F was added to stop the digestion and the separated islet cells were fixed with 70% aqueous ethanol for 30 min at room temperature. The fixed cells were placed on poly-L-lysine-coated glass slides and allowed to air-dry for a few seconds. The islet cells attached to the slide were then washed with 0.05 mol PBS l^{-1} (pH 7.4), islet cell DNA was denatured with 2 mol HCl l^{-1} containing 0.5% (v/v) Triton X-100 for 1 h at room temperature. After washing several times with PBS, the islet cells were incubated with 10% normal goat serum (Chemicon Inc., Temecula, CA) in PBS with 0.1% Triton X-100 for 2 h at room temperature. All of the following procedures were carried out at 4°C . After washing several times with PBS, the islet cells were incubated with a monoclonal mouse anti-BrdU antibody (Chemicon Inc.) for 24 h on a rotating table. The islet cells were washed several times with PBS and were immunostained for BrdU with 1:200 dilution of Rhodamine-conjugated goat anti-mouse immuno-

globulin G (Biosource, Camarillo, CA) in PBS/Triton X for 24 h on a rotating table. After washing several times with PBS, the islet cells were incubated with a diluted polyclonal guinea-pig anti-insulin antibody (ZYMED Lab. Inc., San Francisco, CA) for 24 h on a rotating table and washed several times with PBS. The islet cells were immunostained for insulin with 1:100 dilution of fluorescein isothiocyanate (FITC)-conjugated goat anti-guinea-pig immunoglobulin G (Biomedica Corp., CA) in PBS/Triton X for 24 h on a rotating table. The islet cells were washed several times with PBS and mounted with glycerol-PBS (glycerol:PBS; 7:3) solution.

Measurement of proliferation rate of B cells

The number of B cells incorporating BrdU into their nuclei was determined by direct observation with a Nikon Optiphot microscope equipped for epifluorescence (Nikon Corp., Tokyo). Areas were chosen at random from one side of the slide to the other side. The percentage of B cells with BrdU-labelled nuclei was determined by counting at least 500–1000 FITC-stained B cells for each slide.

Statistical analyses

All the data were expressed as means $\pm$ SEM. Initially, the data from three or more groups were analysed for homogeneity of variance with Bartlett's test. Data meeting this criterion were analysed by one-way analysis of variance and subsequently by Tukey's test. Data showing heterogeneity of variance were analysed initially by the Kruskal–Wallis test and then by Tukey's test. Differences with $P < 0.05$ were considered significant.

Results

Differences in insulin secretion between islets from nonpregnant and pregnant rats

Islets from nonpregnant rats and rats at days 13 and 17 of pregnancy were incubated for 2 h in RPMI-1640 at glucose concentrations of 2.5–10 mmol l⁻¹. Glucose stimulated insulin secretion in the islets of non-pregnant and pregnant rats in a dose-dependent manner (Fig. 1). The secretion of insulin from the islets of rats at day 17 of pregnancy was the greatest, and that from islets of pregnant rats was usually greater than that from the islets of non-pregnant rats.

Effects of trophoblast-culture medium on insulin secretion from islets

Trophoblasts were isolated from the placentae of rats at day 13 of pregnancy and incubated in SFM-101 for 6 days. Islets from nonpregnant rats were incubated for 8 days in SFM containing 0 (control), 10, 50 or 100% of trophoblast-cultured medium and 5% FBS. Trophoblast-culture medium stimulated insulin secretion in a dose-dependent manner (Fig. 2). SFM-101 containing 50 or 100% of trophoblast-culture medium significantly increased insulin secretion compared with that of the

Fig. 1. Effects of pregnancy on insulin secretion at glucose concentrations of 2.5–10 mmol l⁻¹. The islets, obtained from non-pregnant (□), day 13 (◆) or day 17 (●) pregnant rats, were incubated for 2 h in RPMI-1640 at each glucose concentration. In the islets from rats at day 17 of pregnancy, glucose significantly stimulated insulin secretion at concentrations from 2.5 to 10 mmol l⁻¹ compared with non-pregnant rats ($P < 0.01$, $n = 4$). In the islets from rats at day 13 of pregnancy, glucose significantly stimulated insulin secretion at 5 and 10 mmol l⁻¹ compared with non-pregnant rats ($P < 0.05$, $n = 4$).

Fig. 2. Effects of rat trophoblast-culture medium on insulin secretion by non-pregnant rat islets. The non-pregnant rat islets were incubated for 8 days in SFM-101 containing 0 (control), 10, 50 or 100% of trophoblast-culture medium. ** $P < 0.01$ compared with control. Values are expressed per well, and each bar represents mean $\pm$ SEM ($n = 4$).

control medium ($P < 0.01$), and 100% trophoblast-cultured medium doubled insulin secretion by the islets of nonpregnant rats in comparison with the control medium.

Fig. 3. Effects of (●) rat placental lactogen-II (rPL-II), (■) rat prolactin (rPRL) or (△) rat growth hormone (rGH) (1000 ng ml⁻¹) on insulin secretion by islets from (a) non-pregnant rat and (b) rat at day 13 of pregnancy; (○) control. The given values represent the cumulative insulin secretion up to 8 days. In the islets from non-pregnant rats, rPL-II and rPRL significantly stimulated insulin secretion compared with the control on each day and after day 5 of incubation, respectively ($P < 0.01$; $n = 4$). In the islets from rats at day 13 of pregnancy, both rPL-II and rPRL significantly stimulated insulin secretion on each day of the experiment ($P < 0.01$; $n = 4$).

Effects of rPL-II, prolactin and growth hormone on insulin secretion from islets

The islets of non-pregnant rats and rats at day 13 of pregnancy were incubated for 8 days in the presence of 1000 ng ml⁻¹ rPL-II, rPRL or rGH. The insulin concentrations in the culture medium were assayed by radioimmunoassay and the data were expressed daily as a cumulative value. In the experiment using islets from nonpregnant rats, rPL-II significantly stimulated insulin secretion throughout the experiment when compared with the control; when compared with prolactin, there was a significant difference in effects from day 2 of incubation ($P < 0.01$) (Fig. 3). Prolactin significantly stimulated insulin secretion only after day 5 of incubation ($P < 0.01$). Growth hormone tended to cause an increase in insulin secretion, but the effect was not significant. In the experiment using islets from rats at day 13 of pregnancy, rPL-II had a greater effect on insulin secretion than did prolactin, although both hormones significantly stimulated insulin secretion throughout the experiment ($P < 0.01$) (Fig. 3). Growth hormone tended to increase insulin secretion, but again the effect was not significant. The overall pattern of insulin secretion was characterized by a higher magnitude of secretory responses in pregnant rat islets than in those of non-pregnant rats. The dose-response effects of rPL-II on insulin secretion were examined in islets from rats at day 13 of pregnancy. rPL-II stimulated insulin secretion at 50–500 ng ml⁻¹ in a dose-dependent manner with the effect reaching a plateau at 500 ng ml⁻¹ (Fig. 4). The stimulatory effect of rPL-II on insulin secretion was more than double that with rPRL and rGH at the concentration of 100 ng ml⁻¹.

Fig. 4. Dose-response effects of rat placental lactogen-II (rPL-II) on insulin secretion by islets from rats at day 13 of pregnancy. The islets were cultured with the indicated concentrations of hormones for 8 days. Data were cumulated to day 8 of incubation and values are expressed per well as the percentage of the control. rPL-II stimulated insulin secretion in a dose-dependent manner. * $P < 0.01$ compared with rat growth hormone (rGH). ** $P < 0.01$ compared with rat prolactin (rPRL). Each bar represents mean $\pm$ SEM ($n = 4$).

Fig. 5. Double staining for B cells of cultured adult rat islets with 10 mmol 5'-bromo-2'-deoxy-uridine (BrdU) l^{-1} present for the final 24 h of culture. (a) rat B cells immunostained for insulin (green); (b) rat B cells immunostained for BrdU (red); (c) double-staining picture for the B cells immunostained for BrdU (orange) and insulin (light green). The arrows indicate nuclei with incorporated BrdU. Magnification: $\times 890$.

BrdU and insulin immunocytochemistry

The evaluation of proliferating B cells was performed by double-staining for both insulin and BrdU incorporated into replicating DNA. When the B cells were identified using an excitation filter of 450–490 nm, B cells were immunostained as a whole cell body with bright green fluorescence (insulin-positive) (Fig. 5a). However, when the BrdU-incorporated cells were identified using an excitation filter of 510–560 nm, the nuclei with BrdU were immunostained as red fluorescence (BrdU-positive) (Fig. 5b). By using the above two filters, the double-staining picture of both BrdU (orange) and insulin (light green) could be photographed (Fig. 5c). When the anti-BrdU antibody or the anti-insulin antibody was replaced with normal goat serum, no staining was observed in any cells.

Effects of rPL-II, rPRL, and rGH on B-cell proliferation

The effects of rPL-II, rPRL, and rGH on B-cell proliferation was examined by culturing non-pregnant rat islets with rPL-II, rPRL or rGH at 1000 ng ml^{-1} for 8 days including the last 24 h of incubation with 10 mmol BrdU l^{-1} . The rate of proliferation of B cells was expressed as the percentage of the B cells incorporating BrdU of the total B cells counted. rPL-II, rPRL and rGH significantly increased the rate of incorporation of BrdU into the B cells compared with that of the control and their rates were $5.30 \pm 0.36\%$, $3.79 \pm 0.34\%$ and $2.87 \pm 0.29\%$, respectively (Fig. 6). The control had a rate of $1.34 \pm 0.18\%$. The increase in rate induced by rPL-II was found to be approximately four times that of the control.

Discussion

The islet culture system established in the present study is useful of analysing the function of islet cells of pregnant and non-pregnant rats. Insulin secretion from the islets was stimulated by glucose in a dose-dependent manner for concentrations in the physiological range. The secretory response of insulin to the glucose depends on the reproductive process. The response of pregnant rat islets to glucose was greater than

Fig. 6. Effects of rat placental lactogen-II (rPL-II), rat prolactin (rPRL) and rat growth hormone (rGH) at 1000 ng ml^{-1} on incorporation of 5'-bromo-2'-deoxy-uridine (BrdU) into B-cell nuclei. The islets from non-pregnant rats were cultured with 10 mmol BrdU l^{-1} for the last 24 h of culture. Data are shown as mean $\pm$ SEM ($n = 11$ or 12). ** $P < 0.01$ compared with control.

that of non-pregnant rat islets and increased as pregnancy advanced. These results indicate that the glucose stimulation threshold in the islets is reduced and glucose-stimulated insulin secretion is enhanced as pregnancy advances. These functional changes in maternal islets are thought to be needed to accommodate the increased demand of insulin particularly during the last half of pregnancy.

rPL-I enhances islet function (Brelje *et al.*, 1993), which indicates that rat placentae directly regulate insulin secretion during mid-pregnancy in rats. However, as rPL-I appeared in maternal circulation only during mid-pregnancy, the presence of other factor(s) that directly regulate insulin secretion is presumed during the last half of pregnancy. This was verified by adding the culture medium of rat trophoblasts obtained

from the placentae of rats at day 13 of pregnancy to the islet culture system. This led to stimulation of insulin secretion from the islets in a dose-dependent manner. These results suggest that there is a factor(s) that directly regulates insulin secretion in the culture medium of trophoblasts. After mid-pregnancy, rPL-I disappears rapidly from the maternal circulation, and rPL-II is predominantly secreted during the rest of the period. Most of the cells cultured were trophoblast giant cells and polygonal cells obtained from the junctional and labyrinth zones of the chorioallantoic placenta (Kishi *et al.*, 1992). Our previous study confirmed immunohistochemically that approximately 70% of the trophoblast cells are rPL-II positive cells. In addition, the average concentrations of rPL-II in trophoblast-cultured medium are estimated to be more than 300 ng ml^{-1} by radioimmunoassay (data not shown). These findings suggest that rPL-II stimulates insulin secretion directly during the last half of pregnancy.

When purified rPL-II was added to the islet culture system, insulin secretion was stimulated in a dose-dependent manner. rPL-II activity was more than double those of rPRL and rGH at the concentration of 100 ng ml^{-1} in islets from pregnant rats. The PRL concentration in maternal circulation after mid-pregnancy is extremely low in rats except just before parturition (Tonkiewicz and Voogt, 1983), and the GH concentration gradually increases from 50 to 300 ng ml^{-1} in late pregnancy (Kishi *et al.*, 1991). However, the gestational profiles of rPL-II are characterized by a sustained increase in maternal circulation during the last half of pregnancy with a maximum concentration of approximately 1000 ng ml^{-1} (Ogren and Talamantes, 1988; Kishi *et al.*, 1991). Taking into account these three hormone profiles in maternal circulation and our findings, we conclude that rPL-II is a physiological hormone regulating rat islet function during the last half of pregnancy.

When the islets were cultured with 1000 ng ml^{-1} rPL-II, rPRL or rGH for 8 days, islets from pregnant rats displayed a nearly twofold increase in insulin secretion compared with islets from non-pregnant rats. This indicates that the islet function is significantly enhanced during pregnancy. Marynissen *et al.* (1983) have shown that the B-cell mass increased by 53% on day 20 of gestation compared with non-pregnant rats. In addition to changes in the B-cell mass, there are functional changes, including a decreased threshold for glucose stimulation (Sorenson and Parsons, 1985), enhanced glucose-induced insulin secretion (Green and Taylor, 1972), and enhanced insulin synthesis (Bone and Taylor, 1976). These functional changes may be induced both *in vivo* and *in vitro* by the lactogenic hormones prolactin and placental lactogen (Sorenson and Parsons, 1985; Brejle and Sorenson, 1991; Parsons *et al.*, 1992). In pregnant rats, insulin secretion from the maternal islets would increase to harmonize the above changes systemically during pregnancy. The enhanced insulin secretion and increase in B-cell proliferation were correlated with the onset of placental lactogen secretion in the rats (Sorenson and Parsons, 1985; Parsons *et al.*, 1992). These findings suggest that the functional adaptation of B cells is advanced throughout pregnancy by rPRL, rPL-I and rPL-II which are secreted at specific times during each period. Thus, insulin secretion in rat islets would change to match increasing demands for glucose which is indispensable for fetal growth especially during the last half of pregnancy.

The presence of prolactin receptors was demonstrated in rat islet B cells and an increase in expression of prolactin receptors was found in rat islets during pregnancy (Sorenson and Stout, 1995). In the adult mouse liver, mPL-I and mPL-II bind to the same receptor as mPRL (Harigaya *et al.*, 1988), but mouse PLs do not bind to the GH receptors. In rats, one of the gestational hormones, luteotropin, secreted from the decidua binds specifically to rPRL receptors (Gibori *et al.*, 1984). These studies suggest that rPLs use the rPRL receptors in rat tissues. Probably, PRL receptors in B cells would be at least one of the sites to which rPL-II binds during the last half of pregnancy. Although Freemark *et al.* (1994) reported that there is no specific binding site for rPL-II in pregnant rat pancreas, our results suggest cooperativity of binding of rPL-II to unique PL receptors and PRL receptors in maternal B cells, since rPL-II activity was found to be more than double that of rPRL at the concentration of 100 ng ml^{-1} in pregnant rat islets in the present study. The explanation for the observation that rPL-II has more potent activity in islet functions than rPRL remains to be determined.

This study investigated the rate of proliferation of B cells using the thymidine analogue, BrdU, which is incorporated into replicating DNA and detected immunohistochemically by a specific anti-BrdU monoclonal antibody. Although the incorporation of [^3H]thymidine during the S-phase of the cell cycle has been widely used as an index of DNA synthesis and cell proliferation in islets (Logothetopoulos, 1972; Swenne *et al.*, 1980), the islets comprise histologically multiple cell types including four types of endocrine cell and non-endocrine cells such as fibroblasts and endothelial cells (Bonner-Weir and Smith, 1994). The non-endocrine cells have been shown to have much higher thymidine incorporation (Devroede *et al.*, 1990). These findings suggest that the incorporation of [^3H]thymidine into islet cells do not accurately reflect B-cell proliferation. To resolve this problem, we developed a double immunofluorescent staining method to obtain more reliable data on the rate of proliferation of B cells in islets.

B-cell growth is expected to include three aspects: neogenesis of B cells from precursors, changes in the size of individual B cells, and B-cell proliferation (Swenne, 1992). In the present study, it is not clear whether rPL-II affects neogenesis of B cells from precursors and growth in the size of individual B cells. Recent work has suggested that lactogenic hormones induce islet neogenesis and increase islet size (Parsons *et al.*, 1995). The effects of rPL-II on islet neogenesis and growth during pregnancy remain to be determined. Proliferation of existing islet cells occurs late in fetal development and has been thought to be the principal means by which islet mass increases after birth (Hellerstrom *et al.*, 1988). There is a possibility that B-cell replication plays an important role in increasing B-cell mass during pregnancy. The present study suggests that the stimulatory effect of rPL-II on B-cell proliferation is at least one of the mechanisms by which B-cell mass is increased during pregnancy.

Like other stomatic cells, the B cell passes through a cell cycle that can be subdivided into several distinct phases (Hellerstrom *et al.*, 1988). In general, cells must enter an active cycle for proliferation. Most cell populations of islet B cells have been considered to be in an irreversible G_0 phase, i.e. unable to re-enter the cell cycle (Teitelman *et al.*, 1988). In adult

rats, it has been estimated that the proliferative compartment (that is, the fraction of B cells capable of re-entering the cell cycle for 24 h) is less than 3% compared with about 10% in the fetal rat (Swenne, 1983). In the present study, the proliferation rate of B cells in the presence of 1000 ng rPL-II ml⁻¹ for 8 days was 5.3%. This value exceeded the estimated proliferative compartment value of adult rat B cells, suggesting that rPL-II has the potential to increase the estimated proliferative compartment value of adult rat B cells. The proliferative compartment value can be increased by increasing the number of cells re-entering the cell cycle or shortening the duration of the cell cycle. Recently Brelje *et al.* (1994) suggested that rPRL stimulates quiescent B cells to re-enter the cell cycle and divide. This finding points to the possibility that rPL-II stimulates quiescent B cells to re-enter the cell cycle as one of the mechanisms by which it increases the rate of proliferation of B cells during the last half of pregnancy. The effects of rPL-II on the duration of the B-cell cycle remain to be determined.

In the present study, we have demonstrated that rPL-II is a potent stimulator of insulin secretion and B-cell proliferation of islets. This finding is in agreement with the previous finding that mPL-II has the potential to stimulate insulin secretion and B-cell proliferation of mouse islets (Brelje *et al.*, 1993). Moreover, the effects of rPL-II are greater than those of rPRL, which are much greater than those of GH in both insulin secretion and B-cell proliferation. Although PLs, PRL and GH may be regulators of islet function and growth (Nielsen, 1982; Nielsen *et al.*, 1986), it is difficult to interpret these results because of differences in the use of homologous versus heterologous hormones in each study (Brelje *et al.*, 1989). For example, human GH is known to have potency of both somatogenic and lactogenic activities when used with rat tissues (Nicoll *et al.*, 1986). Similar issues may occur within rat PRL-GH families. To avoid these problems, we investigated the effects of rPL-II, rPRL and rGH on islet function in a homologous system and demonstrated that rPL-II is a potent stimulator in rat islet function during the last half of pregnancy. The present study also indicated that rPRL is more effective than rGH for increasing both insulin secretion and B-cell proliferation. These results are in agreement with those of previous studies (Sorenson and Parsons, 1985; Brelje and Sorenson, 1991; Parsons *et al.*, 1992), which suggest that lactogenic hormones have more potent activity than somatogenic hormones in regulating rat islet function.

In conclusion, the present study demonstrates that rPL-II directly regulates insulin secretion and B-cell proliferation of maternal islets, indicating that rPL-II is one of the physiological hormones responsible for increasing islet function during the last half of pregnancy in rats.

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