Adaptation of pancreatic islet B-cells during the last third of pregnancy: regulation of B-cell function and proliferation by lactogenic hormones in rats

Motoyuki Kawai and Kurajiro Kishi
Developmental Research Laboratories, Shionogi and Co. Ltd, Futaba-cho 3-1-1, Toyonaka, Osaka 561-0825, Japan
(Correspondence should be addressed to Motoyuki Kawai, Developmental Research Laboratories, Shionogi and Co. Ltd, Futaba-cho, Toyonaka, Osaka 561–0825, Japan; E-mail: motoyuki.kawai@shionogi.co.jp)

Abstract
In rodents, placental lactogen (PL)-I is considered to be the first trigger to enhance pancreatic islet B-cell function, and after its secretion is diminished at mid-pregnancy, PL-II takes over this role. However, little information is available on the regulation of islet B-cell function and proliferation by lactogenic hormones during the last third of pregnancy. This was the focus of the present study using rats in which pregnancy was forcibly prolonged. This rat possesses unique characteristics in that PL-I is re-secreted during the prolonged period of pregnancy and the peak concentrations in maternal circulation are comparable with those observed during mid-pregnancy in normal-pregnancy rats. Pregnancy was prolonged by successive administration of pregnant mare’s serum gonadotropin (30 IU/rat, s.c. on day 12) and human chorionic gonadotropin (10 IU/rat, i.v. on day 14). When the insulin secretory responses to 10 mmol/l glucose in islets obtained from normal-pregnancy and prolonged-pregnancy rats were tested, each insulin secretory response correlated well with the values of plasma lactogenic activity throughout the period of pregnancy and lactation. Examination of B-cell proliferation in normal-pregnancy rats showed that 5-bromo-2'-deoxyuridine (BrdU) incorporation into dividing B-cells reached a maximum on day 15 and then decreased markedly towards term. No increase in B-cell proliferation was observed on day 19 when plasma lactogenic activity reached the maximum. In prolonged-pregnancy rats, BrdU incorporation also continued to decrease as observed in normal-pregnancy rats after day 15, and then no enhancement in B-cell proliferation was observed even when the plasma lactogenic activity, including re-secreted PL-I, reached maximum. These results suggest that, in the last third of pregnancy, B-cell proliferation is no longer stimulated by lactogenic hormones in contrast to the insulin secretory response which is sustained.

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Introduction
In pregnancy, the insulin demands on the mother dramatically increase due to the enhanced insulin resistance of maternal tissues and increased food intake especially during the latter half of pregnancy. The maternal pancreatic islets are considered to adapt to this increased demand for insulin mainly by enhancing the insulin secretory response and B-cell proliferation (1, 2). Previous studies have suggested that lactogenic hormones secreted during pregnancy are more potent stimulators than somatogenic hormones for inducing functional change of islet B-cells (2–4).

Two major placental lactogens (PLs) have been identified in rodents, one is secreted at mid-pregnancy (PL-I), and the other during the latter half of pregnancy (PL-II). Both PL-I and PL-II are lactogenic hormones rather than somatogenic hormones, interact with prolactin (PRL) receptors, and appear to possess at least some of the same biological actions as pituitary PRL (5–7). Maximum concentrations of both rat (r) PL-I and rPL-II reach approximately 1000 ng/ml during pregnancy (6, 8). rPL-I and rPL-II have strong potencies to induce proliferation in Nb2 lymphoma cells (9). Homologous rPRL stimulates insulin secretion and B-cell proliferation in rat islets (10). Moreover, our previous study demonstrated that homologous rPRL-II stimulates islet B-cell function leading to an increase in the insulin secretory response and the B-cell proliferation in vitro (11). These results suggest that lactogenic activity directly regulates the elevated islet B-cell function during pregnancy in rats.

In rodents, PL-I and PL-II are considered to regulate islet B-cell function during each secretory period. It has been considered that PL-I is the first trigger to enhance B-cell function at mid-pregnancy and PL-II takes over the role of PL-I when its secretion diminishes.
at mid-pregnancy (2). However, little information is available on the regulation of the function and proliferation in islet B-cells by lactogenic hormones during the last third of pregnancy when fetal growth increases markedly. Therefore, we have employed a prolonged-pregnancy rat model in order to understand these phenomena better. A unique characteristic of this rat is that PL-I is re-secreted during the prolonged period of pregnancy and its peak concentrations in maternal circulation are comparable with those observed during mid-pregnancy in normal-pregnancy rats (12, 13).

The present study was undertaken to investigate the regulation of islet B-cell function and proliferation by lactogenic hormones during the last third of pregnancy by comparing prolonged-pregnancy rats with normal-pregnancy rats with respect to insulin secretory response and B-cell proliferation.

Materials and methods

Animals

Female Sprague-Dawley rats (Jcl:SD, CLEA Japan Inc., Ishibe, Japan) weighing 250–350 g were used. On the day of proestrus, each female was housed with a male, and the day sperm were observed in the vaginal smear was designated day 0 (D 0) of pregnancy. In the experiments to estimate islet B-cell proliferation, the animals were lightly anesthetized with ether and implanted s.c. in the dorsal scapular region with eight 50-mg pellets of the thymidine analog 5-bromo-2'-deoxyuridine (BrdU, Boehringer Mannheim Corp., Indianapolis, IN, USA) (3). The implanted pellets were completely dissolved in 24 h.

Rats with new sets of corpora lutea

New corpora lutea were introduced at mid-pregnancy to prolong pregnancy by the method of Shiota et al. (13). Pregnant mare’s serum gonadotropin (PMSG) (30 IU/0.2 ml/rat, s.c.) and human chorionic gonadotropin (hCG) (10 IU/0.1 ml/rat, i.v.) dissolved in 0.85 mol/l NaCl were injected between 1600 and 1700 h on day 12 and between 1600 and 1700 h on day 14 respectively. The treated rats ovulate on day 15 and pregnancy is prolonged. The rats used in this experiment were fasted for 2 h before blood sampling. After light ether anesthesia, blood sampling was performed within one minute from the caudal vena cava between 1000 and 1200 h. The plasma samples were stored at −20°C until assayed for hormones.

Islet isolation and culture

Pancreatic islets were isolated from adult rats by methods reported previously (10). Groups of 25 islets were transferred to 24-well plates (Costar, Cambridge, MA, USA) and incubated in RPMI-1640 medium (Sigma, St Louis, MO, USA) at 37°C under 95% air: 5% CO₂ conditions. The medium was stored at −20°C until assayed for insulin.

Measurement of B-cell proliferation rate

Islet B-cells obtained from BrdU-implanted rats were immunostained for both insulin and BrdU incorporated into the replicating DNA as previously described (11). 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, Japan). The object areas were chosen randomly from one side of the slide to the other side. The percentage of B-cells with BrdU-labeled nuclei was determined by counting at least 500–1000 fluorescein isothiocyanate-stained B-cells for each slide.

Hormone assays

Plasma rPL-II concentrations were determined by RIA as described previously by Kishi et al. (14). Purified rPL-II was obtained from the placenta of day 19 pregnant rats by the method of Kishi et al. (14). Highly purified rPL-II was iodinated by modification of the Iodogen method described by Salacinski et al. (15) using 0.5 mCi carrier-free Na¹²⁵I (Amersham Co., Arlington Heights, IL, USA) and 5 µg hormone. The minimum detectable level of rPL-II was 3.13 ng/ml. Plasma rPL concentrations were determined by RIA with a rat PRL RIA kit provided by the National Hormone and Pituitary Program (Baltimore, MD, USA) and were expressed in terms of NIADDK rat PRL RP-3. The minimum detectable level of rPRL was 0.78 ng/ml. The progesterone concentrations of the plasma samples were determined by RIA with a progesterone RIA kit (Japan DPC Corp., Tokyo, Japan). The insulin concentrations of the plasma samples or the cultured medium were determined by RIA with an insulin RIA kit (Shionogi Co., Ltd, Osaka, Japan).

Estimation of lactogenic activity by radioreceptor assay (RRA)

The lactogenic activity of the plasma samples was estimated by RRA as described previously by Kishi et al. (14). All procedures were carried out at room temperature. Ovine PRL (o-PRL, NIADDK-o-PRL-I-2) was iodinated using 0.5 mCi carrier-free Na¹²⁵I and 5 µg hormone as described above. oPRL (NIADDK-o-PRL-I-17) was used as a standard. The receptors were prepared from mammary gland tissues obtained from lactating New Zealand White rabbits. The minimum detectable level of RRA was 3.13 ng oPRL equivalent/ml plasma.
Statistical analysis
All the data were expressed as means ± S.E.M. Data were analyzed for homogeneity of variance with Bartlett’s test. Data meeting this criterion were analyzed by Tukey’s test. Differences with $P < 0.05$ were considered significant.

Results

Fetal body weights in normal-pregnancy and prolonged-pregnancy rats
The increase of fetal body weights in prolonged-pregnancy rats was similar to that in normal-pregnancy rats until day 21 of pregnancy, and then increased continuously to the extraordinary weight of 7.2 g at day 24 (Fig. 1).

Gestational profiles of plasma insulin in normal-pregnancy and prolonged-pregnancy rats
In normal-pregnancy rats, plasma insulin increased by day 12 and peaked on days 12 to 19 (Fig. 2). After parturition, the values of plasma insulin returned immediately to the non-pregnant rat levels. The overall pattern of the plasma profile of insulin in prolonged-pregnancy rats was similar to that of normal-pregnancy rats. The difference between prolonged-pregnancy rats and normal-pregnancy rats was that the plasma insulin values during prolonged pregnancy remained as high as those observed in the latter half of pregnancy in normal-pregnancy rats.

Gestational profiles of the insulin secretory responses in islets of normal-pregnancy and prolonged-pregnancy rats
In normal-pregnancy rats, the glucose-stimulated insulin secretory responses increased remarkably from day 10 to day 15 and then a small decrease was observed on day 19 (Fig. 3). After parturition, the insulin secretory responses returned immediately to the non-pregnant rat levels. The overall pattern of insulin secretory responses in prolonged-pregnancy rats was similar to that of normal-pregnancy rats. The only difference between the two groups was in the levels observed after day 22 of pregnancy. Namely, the values of the insulin secretory responses observed during the prolonged pregnancy remained relatively high, comparable to that on day 19 of pregnancy. The insulin secretory response observed on day 22 in prolonged-pregnancy rats was significantly higher than that on the corresponding day of lactation (day 1 of lactation) ($P < 0.05$).
Gestational profiles of B-cell proliferation in islets of normal-pregnancy rats and prolonged-pregnancy rats

In normal-pregnancy rats, the rates of B-cell proliferation increased progressively from day 10 to day 15 and then declined markedly to the levels observed in non-pregnant rats until term (Fig. 4). After parturition, the rates of B-cell proliferation remained at the levels observed in non-pregnant rats. The overall pattern of the B-cell proliferation profile in prolonged-pregnancy rats was similar to that of normal-pregnancy rats. There was no significant difference in the rates of B-cell proliferation for the prolonged pregnancy and the corresponding days of lactation (Fig. 4).

Gestational profiles of plasma progesterone, PRL, PL-II and lactogenic activities in normal-pregnancy rats and prolonged-pregnancy rats

In normal-pregnancy rats, plasma progesterone gradually increased to a peak on days 10 to 17 and then decreased markedly until term. In prolonged-pregnancy rats, the profile of plasma progesterone was similar to that in normal-pregnancy rats from day 15 to day 19. Thereafter, the values of plasma progesterone remained relatively high until day 24 (Fig. 4).

Gestational profiles of plasma PRL in normal-pregnancy rats and prolonged-pregnancy rats are shown in Fig. 5B. The values of plasma PRL in prolonged-pregnancy rats remained at basal levels from days 15 to 24. In normal-pregnancy rats, PRL levels were barely detectable from days 6 to 19, but increased dramatically on day 19 and continued to increase to term.

Profiles of plasma PL-II in normal-pregnancy and prolonged-pregnancy rats are shown in Fig. 5C. The overall pattern of the plasma profile of PL-II in prolonged-pregnancy rats is similar to that of normal-pregnancy rats. The only difference was in the plasma levels after day 21. In prolonged-pregnancy rats, the values of plasma PL-II remained relatively high with a decline from days 21 to 24 in contrast to the normal-pregnancy rats in which the values of plasma PL-II decreased markedly to undetectable levels from day 19 to term. The peak values of plasma PL-II in prolonged-pregnancy rats were observed from days 17 to 21 and its concentrations were more than 800 ng/ml.

In normal-pregnancy rats, plasma lactogenic activities showed a biphasic pattern with peaks on days 12 and 19 (Fig. 5D). After day 22 of normal pregnancy, the lactogenic activities were basal values. In prolonged-
pregnancy rats, the profile of the lactogenic activities was similar to that observed in normal-pregnancy rats until day 19. Thereafter, lactogenic activities continued to increase, reached the peak value (approximately 1250 ng/ml) on day 21, and then declined. However, the values after day 21 were significantly higher than those observed in control rats during the corresponding period of lactation ($P < 0.05$).

**Discussion**

The present study showed that the lactogenic activities observed from days 19 to 22 in prolonged-pregnancy rats exceeded those observed on day 19, although the plasma PL-II concentrations decreased markedly in those rats. Plasma PRL values remained at basal levels during the period. These results indicate the presence of lactogenic hormone(s), other than PL-II and PRL, in the maternal circulation during prolonged pregnancy in rats. At present, PL-I is considered to be the only hormone possessing high lactogenic activity among known lactogenic hormones, except for PL-II and PRL (16–20). Therefore, we concluded that PL-I was re-secreted into the maternal circulation during the prolonged period of pregnancy after the introduction of a new set of corpora lutea, although the present study only indirectly confirmed the re-secretion of PL-I during the prolonged period of pregnancy due to the unavailability of the rPL-I RIA system. This finding is also supported by the reports of Shiota and colleagues that PL-I re-secreted during the prolonged period of pregnancy and the peak concentration of PL-I in maternal circulation during the period were comparable with that observed during mid-pregnancy in normal-pregnancy rats (12, 13).

Induction of the PL-I secretory phase during the prolonged period of pregnancy by the introduction of a new set of corpora lutea did not induce B-cell proliferation. This indicates that B-cell proliferation cannot be enhanced by a PL-I stimulus during the prolonged period of pregnancy. PL-I is considered to be the first trigger to enhance B-cell functions at mid-pregnancy (2). In addition, the B-cell proliferation was not enhanced on day 19 of normal pregnancy, when
the maximal peak concentration of PL-II in the maternal circulation was observed. Therefore, most B-cells in the last third of pregnancy may no longer proliferate in response to lactogenic hormones. Several in vivo and in vitro studies have shown that prolonged stimulation of islets by lactogenic hormones can maintain the enhanced islet functions for extended periods (3, 4, 21). These results suggest the possibility that a down-regulation mechanism may exist on the function of islet B-cells in the last third of pregnancy. Sorenson et al. demonstrated that progesterone counterregulates the effects of rat PRL on insulin secretion and B-cell proliferation of rat islets in a homologous in vitro system (22). The pancreatic B-cells in female rats are known to possess progesterone receptor (23, 24). In the present study, the values of plasma progesterone remained relatively high during prolonged pregnancy and on day 19 of normal pregnancy. Therefore, it is likely that the counterregulatory effect of progesterone influences the stimulatory effects of elevated lactogenic activity on B-cell proliferation during the last third of pregnancy.

The present study also showed that B-cell proliferation was enhanced markedly from days 10 to 15 of pregnancy and then decreased remarkably towards term. This gestational profile of B-cell proliferation agrees with that found in the previous study (2). During mid-pregnancy, PL-I is reported to be secreted into the maternal circulation to a maximal concentration of approximately 1000 ng/ml in rats (6). As the first peak of lactogenic activity in maternal circulation was observed at the same period in the present study, this peak is considered to be the secretory profile of PL-I at mid-pregnancy. Rat, mouse and human PL-Is have been found to stimulate B-cell proliferation in the islets when examined using homologous systems (10). Thus, the time-specific stimulus of PL-I at mid-pregnancy may be important for adequate B-cell proliferation during pregnancy in the rat.

The values of the glucose-stimulated insulin secretory response in the islet B-cells remained at relatively high levels in the presence of high lactogenic activity in maternal circulation during the last third of pregnancy and prolonged pregnancy, although no enhancement of B-cell proliferation was observed during these periods. The insulin secretory response observed on day 22 in prolonged-pregnancy rats was significantly higher than that on the corresponding day of lactation (day 1 of lactation), when placental lactogens have already disappeared in the maternal circulation. We also observed a good correlation between the profile of the insulin secretory response and that of the lactogenic activity in maternal circulation during these periods, including the period of lactation. These results suggest that the lactogenic hormones continue to regulate the insulin secretory response but not B-cell proliferation during the last third of pregnancy. However, the present findings could not explain the reason for the different actions on insulin secretion and B-cell proliferation observed during prolonged pregnancy. One possible explanation is the diversity of the lactogenic receptor signaling in B-cell function. Several analyses of the growth hormone (GH) receptor in B-cell function have demonstrated that the proliferative signaling only requires a membrane proximal part of the receptor and activation of the tyrosine kinase Janus activation kinase 2 and the transcription factors STAT1 and STAT3 (signal transducers and activators of transcription) (25). On the other hand, the action of insulin secretion via the GH receptor requires the distal part of the receptor and activation of calcium uptake and STAT5 (26–28). Thus, GH (and PRL) may affect insulin secretion and B-cell proliferation via distinct signaling pathways. Therefore, the counterregulatory effect of progesterone on the effects of lactogenic hormones may be more effective on B-cell proliferation than on insulin secretion during the last third of pregnancy.

An alternative mechanism to account for the different actions of the insulin secretion and the B-cell proliferation during prolonged pregnancy is the existence of other specific factor(s) implicated in B-cell function. The hepatocyte growth factor was found to stimulate proliferation of adult human islet B-cells (29), but to reduce the insulin content in fetal human islet B-cells (30). In addition, nicotinamide has been reported to inhibit B-cell proliferation but to enhance insulin production on an extra-cellular matrix (31, 32). Therefore, we cannot exclude the possibility that the prolonged pregnancy condition changes the gestational profiles of several factors implicated in B-cell function during prolonged pregnancy. Further studies will be necessary to explain the different actions of the insulin secretion and the B-cell proliferation during prolonged pregnancy.

The fetal body weights showed a linear increase during prolonged pregnancy, and the maternal plasma insulin concentrations during the period remained high, comparable with those of normal-pregnancy rats in the last third of pregnancy. As fetal growth is dependent on an adequate glucose supply from the mother, maternal islets are thought to be needed to accommodate the increased demand for glucose particularly during the last third of pregnancy when fetal growth is remarkable (33). Therefore, the present study suggests that PL-II, which is considered to be the main lactogenic hormone secreted during the last third of pregnancy, helps maintain maternal glucose metabolism during this period mainly through continuous enhancement of the insulin secretory response in B-cells and not enhancement of B-cell proliferation.

We conclude that, in the last third of pregnancy, B-cell proliferation is not continually stimulated by lactogenic hormones in contrast to the insulin secretory response, which is maintained.
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References


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