Role of cAMP in Upregulation of Insulin Secretion During the Adaptation of Islets of Langerhans to Pregnancy

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Islets undergo a number of upregulatory changes to meet the increased demand for insulin during pregnancy, including an increase in glucose-stimulated insulin secretion with a reduction in the stimulation threshold. Treatment with the lactogenic hormone prolactin (PRL) in vitro has been shown to induce changes in islets similar to those observed during pregnancy. We examined cAMP production in islets treated with PRL to determine if changes in cAMP are involved in the upregulation of insulin secretion. Insulin secretion and cAMP concentrations were measured from islets in response to a suprathreshold (6.8 mmol/l) or high (16.8 mmol/l) glucose concentration in the presence of the phosphodiesterase inhibitor isobutylmethylxanthine. Insulin secretion increased by 2.1-, 5.0-, and 5.9-fold at the suprathreshold glucose concentration and by 1.6-, 2.3-, and 2.9-fold at the higher glucose concentration after 1, 3, and 5 days of PRL treatment, respectively. After a similar pattern, cAMP metabolism increased by 1.2-, 1.6-, and 2.1-fold at the suprathreshold glucose concentration and by 1.2-, 1.7-, and 2.2-fold at the high glucose concentration after 1, 3, and 5 days of PRL treatment, respectively. The similar increases in insulin secretion and cAMP concentration suggest that changes in cAMP metabolism are involved in lactogen-induced upregulation of insulin secretion. To gain additional insight into the role of cAMP in the upregulation of islet function after lactogen treatment, we examined the relationship between changes in cAMP concentration and insulin secretion. Under all conditions (differing glucose concentrations and time periods), the increase in insulin release was directly proportional to the increase in cAMP. Thus increased glucose-stimulated insulin secretion from lactogen-treated islets could be accounted for by increased generation of cAMP and did not appear to require any further specific changes in intracellular processes mediated by cAMP. Because the PRL receptor is not directly involved in cAMP metabolism, the lactogen-induced increase in cAMP was most likely due to the increase in glucose metabolism that we have previously demonstrated in PRL-treated islets and in islets during pregnancy. *Diabetes* 47:1426–1435, 1998

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IBMX, isobutylmethylxanthine; KRB, Krebs-Henseleit solution; PRL, prolactin.

To accommodate the increased demand for insulin that occurs during pregnancy, the islets of Langerhans undergo major structural and functional changes. Adaptive changes that occur include *1*) increased glucose-stimulated insulin secretion with *2*) a decrease in the glucose-stimulation threshold (1–3), *3*) increased insulin synthesis (4,5), and *4*) increased glucose utilization and oxidation (3,6). An important feature of islets as they adapt to pregnancy is that they do so over a long period of time—for example, it takes up to 5 days in rats for the islets to fully adapt (7).

In vitro and in vivo experiments in which the effects of homologous prolactin (PRL) or placental lactogen on islets have been examined have indicated that hormones of lactogenic specificity induce the same changes in islets as those observed during pregnancy. These changes include *1*) enhancement of glucose-stimulated insulin secretion (8,9), *2*) decreased glucose stimulation threshold (8,9), *3*) increased insulin synthesis (10), and *4*) increased glucose utilization and oxidation (3,6). Furthermore, the changes observed in lactogen-treated islets require a similar length of time to occur as those observed in pregnancy. Based on these observations, it is apparent that lactogens (placental lactogen and/or PRL) are the key regulatory hormones in islet adaptation to pregnancy.

Increased cAMP metabolism has long been suspected to be a mechanism involved in potentiating insulin secretion and lowering the stimulation threshold. In studies examining islets from pregnancy that exhibited upregulated glucose-stimulated insulin secretion, it was found that adenylyl cyclase activity and cAMP levels were elevated (5,11). Furthermore, we have previously shown that insulin secretory profiles from perfused pancreases in response to glucose were altered in the presence of forskolin or glucagon, agents known to increase intracellular cAMP levels in β-cells (12). These secretory profiles share two important characteristics with those obtained from glucose-stimulated pregnancy islets or PRL-treated islets: an increase in the magnitude of insulin secretion and a shift in the stimulation threshold to lower glucose concentrations. In addition, studies have shown that transgenic mice with constitutive expression of the active mutant α_s subunit of the G_s-protein in their islet β-cells have increased cAMP levels with an elevated insulin secretory response in the presence of the phosphodiesterase inhibitor isobutylmethylxanthine (IBMX) (13). Other studies have shown that increasing cAMP levels in islet β-cells with membrane-permeable cAMP analogs (db-cAMP

and $[Bu]_2$cAMP) as well as with IBMX or glucagon lead to the enhancement of glucose-stimulated insulin release with a decrease in the stimulation threshold (14–17). Furthermore, it has been shown that the dissociation of islets to isolated β-cells considerably lowers nutrient-induced insulin secretion (14), a finding that has been attributed primarily to the low cAMP concentrations in these cells, as their secretory ability is subsequently restored when the cAMP levels are increased (14).

There is ample evidence that in the absence of or at low concentrations of glucose, the augmentation of cAMP content has little if any effect on insulin secretion (19,21). Furthermore, the augmentation of secretion by cAMP occurs only above the concentration of glucose sufficient to stimulate secretion independently (17–19). The accepted threshold for the stimulation of insulin secretion by glucose is 5.6 mmol/l in normal rats. This glucose concentration is also the accepted threshold for the stimulation of cAMP metabolism in islet β-cells (17,18). These results thus support the concept that upregulation of cAMP is involved, and perhaps required, for the potentiation of insulin secretion at stimulating glucose concentrations. This suggests that cAMP cannot be considered as the essential second messenger mediating the secretory action of glucose, but rather amplifies or potentiates the extent of insulin secretion. The precise role of cAMP in the complex process of potentiating insulin release is poorly understood. However, it is known that glucose stimulation of islets produces a marked elevation of cAMP levels (17,20–22). The dependence of glucose metabolism on cAMP metabolism was further demonstrated by incubating islets with mannoheptulose, an inhibitor of glycolysis, which caused the inhibition of glucose-stimulated cAMP metabolism and insulin secretion (22,23). These observations support the idea that cAMP is a potentiator rather than an initiator of insulin secretion.

The mechanisms by which glucose increases cAMP metabolism are largely unknown. Studies have shown that glucose has no direct effect on adenylyl cyclase activity in islet homogenates (19). However, because islet adenylyl cyclase is activated by Ca^{2+}-calmodulin, it has been proposed that glucose's effect in increasing cAMP metabolism in intact islets is mediated by Ca^{2+}-calmodulin and, hence, is secondary to an increase in cytosolic Ca^{2+} concentration $[Ca^{2+}]_i$ (24,25).

A number of studies have been conducted to describe the mechanisms by which an increase in cAMP may lead to the potentiation of insulin secretion. Increased rates of phosphorylation of ion channels in the islet β-cell membrane via cAMP-dependent protein kinase A may be a mechanism leading to increased β-cell sensitivity to primary stimuli (15,26–28). This process results in more rapid membrane depolarization; increased Ca^{2+} influx through voltage-dependent channels, which thereby elevates $[Ca^{2+}]_i$; and accelerated exocytosis. In addition, cAMP has also been shown to promote insulin release by an interaction distal to regulatory steps of the secretory machinery (27,29–31). It is important to note that these effects of cAMP lead to the potentiation of insulin secretion, a major characteristic of lactogen-induced upregulation of insulin secretion.

In this study, we examined the effect of lactogens on insulin secretion and islet cAMP metabolism in vitro with PRL treatment and in vivo during pregnancy to test our hypothesis that lactogens increase islet cAMP metabolism and that these increased levels are involved in the enhancement of the magnitude of insulin secretion and the shift of the stimulation threshold to a lower glucose concentration.

RESEARCH DESIGN AND METHODS

Islet isolation and cell culture. For experiments using islets treated with PRL in vitro, rat islets were isolated from neonates ages 3–6 days pooled from two or more litters of SD rats (Harlan Sprague-Dawley, Indianapolis, IN) by a nonenzymatic method previously described (32). After isolation, groups of 25 islets were transferred to 24-well plates (Costar, Cambridge, MA) and cultured free-floating in 2 ml RPMI 1640 containing 10 mmol/l glucose supplemented with 10% horse serum (HyClone Technologies, Logan, UT), 10 mmol/l HEPES, and 1% penicillin-streptomycin-fungizone antibiotic-antimycotic (Sigma, St. Louis, MO). PRL was used at a dosage of 500 ng/ml, and the culture medium was changed daily during the 5-day treatment period. Rat prolactin (rPRL B-8-SIAFP; 30 IU/ mg) was obtained from the National Hormone and Pituitary Program of the National Institute of Diabetes and Digestive and Kidney Diseases (Baltimore, MD).

For experiments using islets from pregnancy, islets were isolated from timed pregnant SD rats on day 15 of pregnancy by pancreatic distension with a collagenase solution followed by stationary in vitro digestion (33). Islets were then purified on a discontinuous ficoll gradient. After isolation, a sample of islets was routinely stained with propidium iodide (1 µg/ml; Sigma) as a test of viability. The islets were then preincubated for 1 h at 37°C in RPMI 1640 (Sigma) plus 10% horse serum (HyClone) and 2 mmol/l glucose before being used for experiments.

Insulin secretion. In all of the in vitro PRL-treatment experiments, daily insulin secretion was monitored in the culture medium. This was done to determine steady-state secretion and to ensure that there was a full effect of PRL treatment on the tissue used for further analysis. Insulin secretion was also examined after acute stimulation with 6.8 or 16.8 mmol/l glucose for 1 h. Insulin was measured in samples by radioimmunoassay (34) and expressed as $mU \cdot islet^{-1} \cdot 24\ h^{-1}$ from islets during steady state and as $mU \cdot islet^{-1} \cdot h^{-1}$ from acute glucose-stimulated islets.

Measurement of cAMP content. For the measurement of steady-state cAMP production during PRL treatment in vitro, neonatal islets in batches of 50 were examined directly from culture after 1, 3, and 5 days. For the measurement of acute glucose-stimulated cAMP production, batches of 25 neonatal islets were examined after being taken from culture after 1, 3, or 5 days of treatment. Batches of 25 adult islets were examined after a preincubation period of 1 h.

These islets were then incubated for 60 min in buffered Krebs-Henseleit solution (KRB) composed of 120 mmol/l NaCl, 4.8 mmol/l KCl, 2.6 mmol/l $CaCl_2$, 1.18 mmol/l KH_2PO_4, 1.1 mmol/l $MgSO_4$, 25 mmol/l $NaHCO_3$, 10 mmol/l HEPES, and 0.1% bovine serum albumin containing 2.8 mmol/l glucose (37°C, 5% CO_2, humidified) to allow islets to reach baseline metabolic rates. Insulin secretion and cAMP concentrations were then examined after incubation in 6.8 or 16.8 mmol/l glucose for 1 h in the presence of 500 µmol/l of the phosphodiesterase inhibitor IBMX (Sigma).

The islets were washed in 1.7 ml Eppendorf tubes with 1 ml Hank's balanced salt solution at 4°C containing 500 µmol/l IBMX (to block the degradation of cAMP) (18,22) and centrifuged at 800*g*; the supernatant was then discarded. Islets were quick-frozen in an ethanol/dry ice bath. The supernatant left with the islets during the separation was negligible. Islets were stored frozen at –80°C until the time of assay.

To extract cAMP for measurements, islets were disrupted by sonication for 5 sec in 500 µl 95% ethanol at 4°C, vortexed vigorously, and centrifuged at 15,000 rpm for 30 min at 4°C (35). The supernatant was removed and evaporated to dryness. Samples were redissolved in sodium acetate buffer (0.05 mol/l; pH 6.2), and cAMP levels were determined using an enzyme-linked immunosorbent assay kit for cAMP (TiterZyme, dual range cAMP enzyme immunoassay; PerSeptive Biosystems, Cambridge, MA) in 96-well plates on a spectrophotometer (Multiskan; Titertek, Huntsville, AL) at 405 nm. The detection range of the assay was 100–6,000 fmol. Each assay included quality controls containing cAMP standard and a positive control containing 10 µmol/l forskolin, a stimulator of adenylyl cyclase in islets that increases cAMP levels above controls more than 10-fold, as previously described (28). cAMP content is expressed as fmol/islet.

Expression of data and statistical methods. All values are expressed as means ± SE of *n* separate observations. Statistical differences between means were assessed with Student's *t* test for unpaired samples.

RESULTS

Effect of PRL treatment on insulin secretion. To measure the effect of PRL treatment on insulin secretion, media samples were taken daily from islets in culture for 5 days. PRL treatment increased secretion above control values (over a period of 24 h in the presence of 10 mmol/l glucose) (Fig. 1).

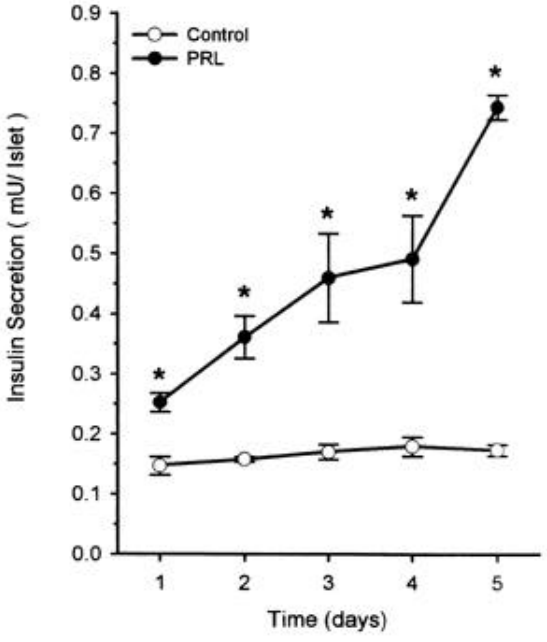

FIG. 1. Effect of 500 ng/ml PRL treatment on insulin secretion from cultured rat islets over 5 days in the presence of 10 mmol/l glucose. The medium was changed daily and 24-h insulin secretion was determined. PRL treatment resulted in a significant increase in insulin secretion compared with in control islets at all time points. *P < 0.05; n = 4.

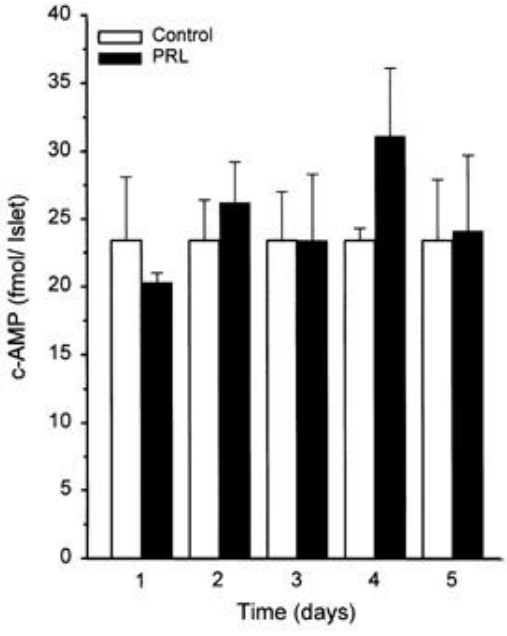

FIG. 2. Effect of 500 ng/ml PRL treatment in vitro on steady-state cAMP levels over 5 days. The islets were cultured as described in Fig. 1 and taken from culture at the indicated time; cAMP concentrations were then measured. No changes in cAMP concentrations were observed during the 5 days of PRL treatment (n = 3).

Insulin secretion was increased 1.8-fold after 1 day (P < 0.05; n = 4), 2.6-fold after 3 days (P < 0.05; n = 4), and 4.5-fold after 5 days (P < 0.01; n = 4). Note that although a significant increase is often observed after 1 day of treatment, it requires 3–5 days for PRL to have its maximal effect on insulin secretion (3,8,9).

Effect of PRL treatment on steady-state cAMP levels. To examine the role of cAMP in the upregulation of insulin secretion after PRL treatment, steady-state cAMP levels in control and PRL-treated islets were measured during the 5 days of culture. The cAMP concentration was measured immediately after removal of the islets from the culture medium containing 10 mmol/l glucose. The cAMP concentrations were 20–25 fmol/islet (n = 3) for both control and PRL-treated islets (Fig. 2). No change in cAMP concentration was observed during the 5 days of PRL treatment. These values are similar to those previously reported in the literature for isolated rat islets (10,18,20,36). These data show that PRL-induced changes in insulin secretion were not mediated by changes in the steady-state level of cAMP.

Effect of PRL treatment on glucose-stimulated insulin secretion and cAMP production. To obtain a better measurement of the potential effects of PRL on cAMP metabolism, cAMP concentrations were examined in islets after 1 h of glucose stimulation. It has been previously shown that in the absence of or at low concentrations of glucose, cAMP has little if any effect on secretion. The potentiation of insulin secretion by cAMP occurs only above the concentration of glucose sufficient to stimulate secretion independently. The accepted glucose-stimulation threshold is 5.6 mmol/l in rats (12,17,18,37). Similarly, this concentration is also the accepted threshold for the stimulation of cAMP metabolism in β-cells. Therefore, in the subsequent experiments we examined insulin secretion at a slightly stimulatory concentration of 6.8 mmol/l (suprathreshold) and a high glucose concentration of 16.8 mmol/l. Insulin secretion at 6.8 mmol/l glucose should be considerably enhanced from PRL-treated islets because of the lowering of the threshold for glucose-stimulated insulin secretion (12). At 16.8 mmol/l glucose, the results should demonstrate the role of cAMP in increasing the magnitude of insulin secretion at high glucose concentrations, also a characteristic of PRL treatment.

The addition of the phosphodiesterase inhibitor IBMX (500 µmol/l) has been shown in previous studies to inhibit the breakdown of cAMP and allow the measurement of islet cAMP production in the absence of catabolism (5,13,18,22). In our experiments, this technique would accentuate the differences that occur in cAMP production as a result of PRL-induced upregulation. However, this would also obscure any differences in cAMP breakdown.

Insulin secretion and cAMP concentrations were examined in islets after 1, 3, and 5 days in culture. The islets were preincubated in 2.8 mmol/l glucose for 1 h, and then stimulated with 6.8 or 16.8 mmol/l glucose in the presence of 500 µmol/l IBMX. In response to 6.8 mmol/l glucose, the cAMP concentration in control islets was 30.6 ± 1.9 fmol/islet (n = 4) (Fig. 3). For all conditions examined, an increase in cAMP concentration was observed between the 6.8 mmol/l and 16.8 mmol/l glucose trials (Figs. 3 and 4). A cAMP concentration of 39.3 ± 1.7 fmol/islet (n = 4) was observed for control islets in response to 16.8 mmol/l glucose (Fig. 4). These data are in agreement with previously reported values for rat islets incubated in the presence of IBMX (5,11,14).

As expected from our previous results, insulin secretion in response to 6.8 mmol/l glucose for 1 h was significantly increased above that in control islets after 1 day of PRL treatment (Fig. 3). Insulin secretion was increased by 2.1-fold (P

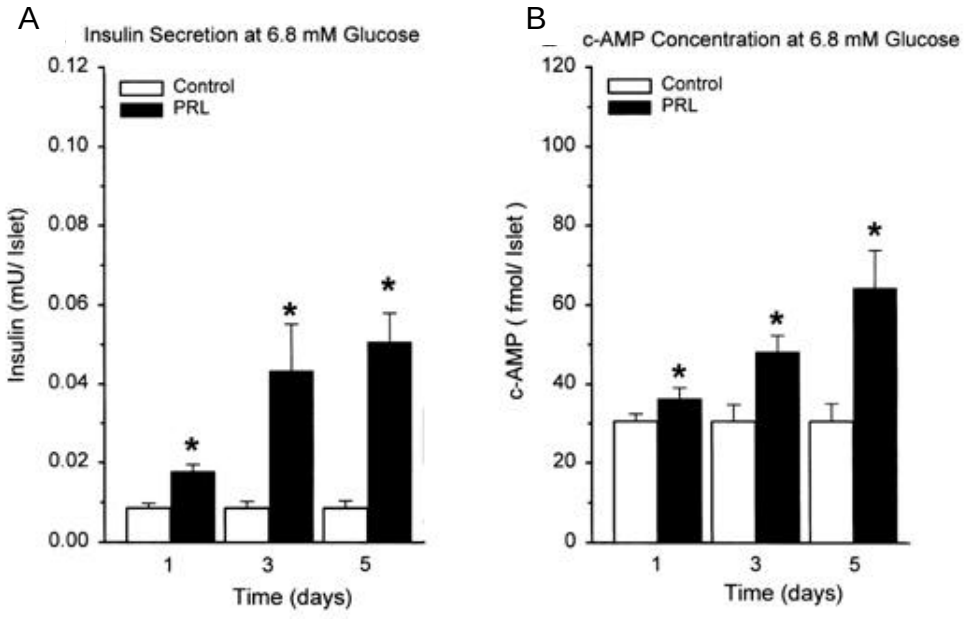

FIG. 3. Effect of 500 ng/ml PRL treatment in vitro on insulin secretion (A) and cAMP concentration (B) from rat islets in response to an acute stimulation with a suprathreshold glucose concentration. Islets were taken from culture after 1, 3, or 5 days of treatment, incubated for 60 min in KRB containing 2.8 mmol/l glucose, and then stimulated for 1 h in KRB containing 6.8 mmol/l glucose and 500 µmol/l IBMX. In response to a suprathreshold (6.8 mmol/l) glucose concentration, PRL treatment significantly increased both insulin secretion and cAMP concentrations above control values at all time points examined. *P < 0.05; n = 4.

< 0.05; n = 4), 5.0-fold (P < 0.05; n = 4), and 5.9-fold (P < 0.05; n = 4) after 1, 3, and 5 days of treatment, respectively. Correspondingly, cAMP concentration was increased by 1.2-fold (P < 0.05; n = 4), 1.6-fold (P < 0.05; n = 4), and 2.1-fold (P < 0.01; n = 6) after 1, 3, and 5 days of treatment, respectively. These data show that PRL treatment increased both insulin secretion and cAMP concentration above control levels after 1 day of treatment, and continued to increase both through day 5. The corresponding increases in insulin secretion and cAMP concentration suggest that cAMP was involved in the enhancement of secretion after PRL treatment.

At the higher concentration of 16.8 mmol/l glucose, insulin secretion was significantly increased in PRL-treated islets compared with control islets (Fig. 4). These increases were 1.6-fold (P < 0.05; n = 4), 2.3-fold (P < 0.05; n = 3), and 2.9-fold (P < 0.01; n = 3) after 1, 3, and 5 days of PRL treatment, respectively. Similarly, cAMP concentrations were increased by 1.2-fold (P < 0.01; n = 4), 1.7-fold (P < 0.05; n =

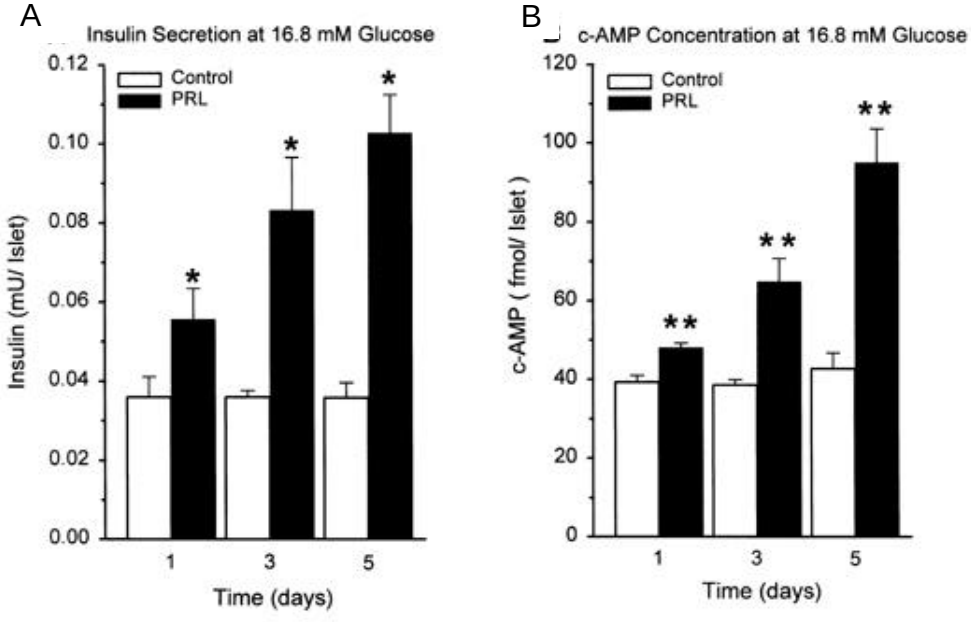

FIG. 4. Effect of 500 ng/ml PRL treatment in vitro on insulin secretion (A) and cAMP concentration (B) from rat islets in response to an acute stimulation with a high glucose concentration. Islets were treated as described in Fig. 3, but stimulated for 1 h in KRB containing 16.8 mmol/l glucose and 500 µmol/l IBMX. In response to a high (16.8 mmol/l) glucose concentration, PRL treatment significantly increased both insulin secretion (*P < 0.05; n = 4) and cAMP concentrations (P < 0.01; n = 4) above control values at all time points examined.**

TABLE 1
Effect of pregnancy on insulin secretion and cAMP concentration in response to an acute stimulation with a suprathreshold (6.8 mmol/l) or high (16.8 mmol/l) glucose concentration

	Insulin (mU/islet)		cAMP (fmol/islet)	
	Control	Day 15	Control	Day 15
6.8 mmol/l	0.031 ± 0.007	0.354 ± 0.066*	31.6 ± 6.8	51.2 ± 6.1
16.8 mmol/l	0.165 ± 0.028	0.724 ± 0.097*	53.4 ± 3.1	97.7 ± 10.5*

Islets were isolated from day-15 pregnant and control rats, incubated for 60 min in KRB containing 2.8 mmol/l glucose, and then stimulated for 1 h in KRB containing 6.8 or 16.8 mmol/l glucose and 500 µmol/l IBMX. In response to 6.8 mmol/l glucose, insulin secretion was significantly increased in pregnant islets ($P < 0.05$, $n = 3$), and cAMP was marginally increased by 1.6-fold ($P = 0.08$, $n = 3$). In response to 16.8 mmol/l glucose, both secretion and cAMP were significantly increased in pregnant islets ($P < 0.01$, $n = 3$, and $P < 0.05$, $n = 3$, respectively). *Statistical significance when compared with controls.

4), and 2.2-fold ($P < 0.001$; $n = 6$) after 1, 3, and 5 days of PRL treatment, respectively (Fig. 4). Similar to the data at the 6.8 mmol/l glucose level, the corresponding increases in insulin secretion and cAMP concentration suggest that they are correlated with each other.

Effect of pregnancy on acute glucose-stimulated insulin secretion and cAMP production. To confirm the in vitro data, insulin secretion and cAMP concentration were measured in isolated islets from pregnant and control rats after 1 h of glucose stimulation. From our previous studies (2,7), we know that the largest increase in insulin secretion is observed on day 15 of pregnancy.

For the measurement of insulin secretion and cAMP concentrations, control and 15-day pregnant islets were preincubated in 2.8 mmol/l glucose and then stimulated with 6.8 or 16.8 mmol/l glucose in the presence of 500 µmol/l IBMX. In response to 6.8 mmol/l glucose, cAMP levels in control islets were 31.6 ± 6.8 fmol/islet ($n = 4$) (Table 1). Raising the glucose concentration in control islets to 16.8 mmol/l significantly increased the cAMP concentration 1.6-fold above the response at 6.8 mmol/l ($P < 0.05$; $n = 3$), thereby demonstrating an increase in cAMP production in adult islets after the elevation of the glucose concentration.

In response to 6.8 mmol/l glucose, insulin secretion in islets from pregnant rats was significantly increased above that in control islets. Insulin secretion was increased 11.4-fold ($P < 0.05$; $n = 3$) on day 15 of pregnancy (Table 1), whereas the cAMP concentration was increased by 1.6-fold ($P = 0.08$; $n = 3$) (Table 1). These observations are similar to those shown for the PRL-treated islets in vitro.

In response to 16.8 mmol/l glucose, insulin secretion was significantly increased in islets from pregnant rats by 4.4-fold ($P < 0.01$; $n = 3$) (Table 1). Similar to the observations of islets treated with PRL in vitro, cAMP concentrations in response to 16.8 mmol/l glucose were also significantly increased by 1.8-fold ($P < 0.05$; $n = 3$) in islets from pregnant rats (Table 1). These observations are similar to those shown for PRL-treated islets in vitro.

These observations support our hypothesis that lactogen-treatment of islets leads to increased cAMP metabolism. This increased metabolism is involved in the potentiation of glucose-stimulated insulin secretion, contributing to the overall enhancement of secretion observed in upregulated islet function after lactogen treatment.

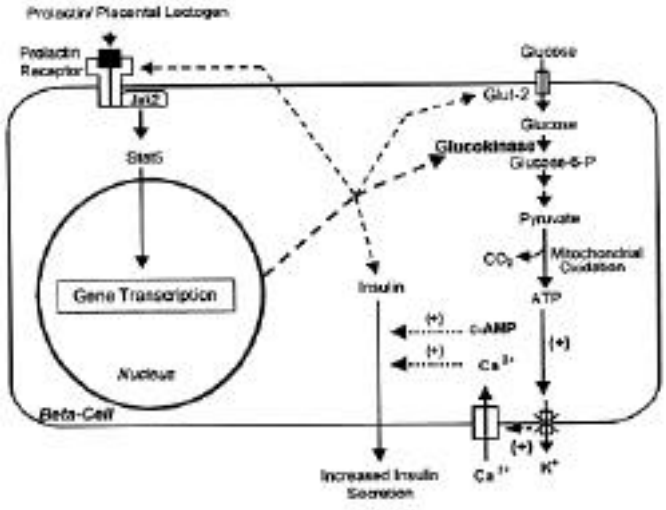

FIG. 5. A model of the mechanisms by which pregnancy or PRL leads to upregulation of islet β-cell function. Lactogens directly upregulate the expression of PRL receptor and glucokinase genes, leading to enhanced glucose metabolism as well as increased expression of GLUT2 and insulin. This in turn leads to increased rates of glucose utilization and oxidation, as well as the enhancement of intracellular pathways that can potentiate insulin secretion, including cAMP. Glucose-6-P, glucose-6-phosphate; Jak2, Jak tyrosine kinase 2; Stat5, signal transduction and activator of transcription 5.

DISCUSSION

The general features of islets as they adapt to pregnancy have become increasingly better characterized. The primary changes that occur in islets during pregnancy are enhanced glucose-stimulated insulin secretion with a reduction of the threshold to lower glucose concentrations (38,39). There is now strong evidence that these changes are brought about by activation of the PRL receptor on islets by placental lactogens present during pregnancy in rodents (38,39). Results from both in vitro and in vivo experiments in which the effects of homologous PRL or placental lactogen on islets have been examined have indicated that hormones of lactogenic specificity induce similar changes to those observed during pregnancy.

Our previous studies have suggested that the upregulation of glucose metabolism has a primary role in the enhancement of insulin secretion observed after the exposure of islets to PRL (3). According to our hypothesis (Fig. 5), lactogens upregulate the PRL receptor and glucokinase, leading to enhanced glucose metabolism. This acts to increase expression of GLUT2 and insulin, as well as increase the rates of glucose utilization and oxidation. A likely result of this increased metabolism is the enhancement of other intracellular pathways that can potentiate insulin secretion. However, it is unclear whether lactogen treatment has additional direct effects on these potentiation pathways.

In the present study, we focused on changes in cAMP levels to determine whether alterations in cAMP metabolism are involved in lactogen-induced enhancement of insulin secretion in islets. Insulin secretion and cAMP concentrations were examined in islets after 1, 3, and 5 days in culture. The data show that in response to suprathreshold (6.8 mmol/l)

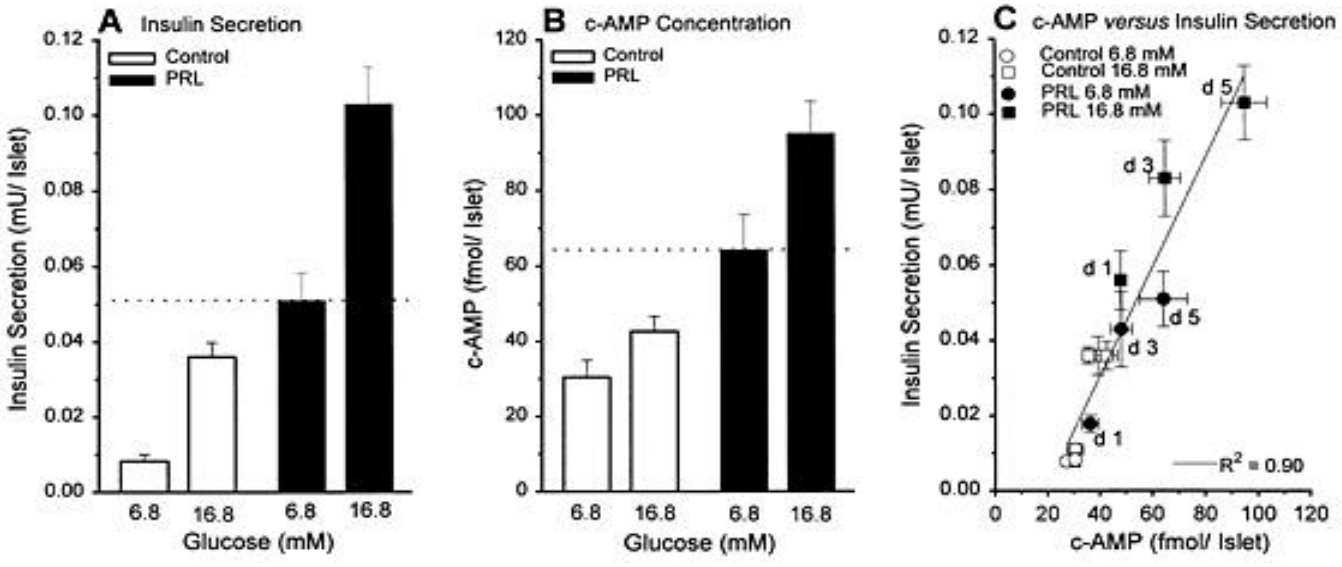

FIG. 6. Insulin secretion (*A*) and cAMP concentrations (*B*) from cultured islets after 5 days of PRL treatment in response to an acute stimulation with a suprathreshold (6.8 mmol/l) or high (16.8 mmol/l) glucose concentration in the presence of 500 µmol/l IBMX. Both insulin secretion and cAMP concentrations from PRL-treated islets in response to a slightly stimulatory (6.8 mmol/l) glucose concentration (- - -) were higher than those from control islets in response to high (16.8 mmol/l) glucose. *C*: Correlation between insulin secretion and cAMP concentrations from control and PRL-treated islets after 1, 3, and 5 days of PRL treatment. In all cases, the increase in secretion was proportional to the increase in cAMP, as visualized by a linear relationship (r^2 = 0.90), regardless of whether the data were from PRL-treated or control islets.

and high (16.8 mmol/l) glucose stimulation, both insulin secretion and cAMP production were increased above control levels after 1 day of PRL treatment or by day 15 of pregnancy. In all cases, the increase in insulin secretion was paralleled by a similar increase in cAMP concentration. These results support previous studies that observed an increase in adenylyl cyclase and protein kinase A activity in islets during pregnancy (5,11,40). A major characteristic of lactogen-treated islets is that they require a long time to fully adapt. In the present study, the kinetics of upregulation of cAMP occurred gradually over a period of 5 days, suggesting it is involved in this long-term adaptation. It is important to note that although lactogen treatment requires activation of the PRL receptor, this receptor is not directly coupled to cAMP metabolism. This is in contrast to rapid changes (within minutes) in cAMP metabolism mediated by hormone receptors that stimulate adenylyl cyclase by way of G-proteins. This suggests that lactogen-treatment leads to an increase in cAMP metabolism through an indirect mechanism related to the long-term upregulation of islet function.

To gain insight into the role of cAMP in the upregulation of islet function after lactogen treatment, we further examined the correlation between the changes in cAMP and insulin secretion. For example, we compared the response of islets to suprathreshold (6.8 mmol/l) and high (16.8 mmol/l) glucose concentrations after 5 days of PRL treatment to the response observed in control islets (Fig. 6*A* and *B*). By this time point, the cAMP concentration and insulin secretion at this slightly stimulatory glucose concentration were higher than those observed with the high glucose concentration from control islets. The correlation of insulin secretion to cAMP levels for all the time points examined is shown in Fig. 6*C*. In all cases, the increase in insulin release was proportional to the increase in cAMP. Furthermore, a linear relationship (r^2 = 0.90) between cAMP and insulin secretion was observed, even though the data were from different islet populations (i.e., control islets and islets after 1, 3, and 5 days of PRL treatment). Although not shown, the data from adult islets isolated from pregnant and control rats also demonstrated a strong linear correlation (r^2 = 0.94), which indicates that the increased insulin secretion from PRL-treated islets might be accounted for by the increased generation of cAMP throughout the adaptive process. It also suggests that the intracellular processes mediated by cAMP (i.e., those distal to the production of cAMP that can potentiate the rate of insulin release) are unchanged during the adaptation of islets to PRL. In addition, the increased insulin secretion from PRL-treated islets might be accounted for by the increased generation of cAMP under these conditions.

The existence of a linear relationship between cAMP levels and the rate of insulin secretion has been previously described by Katada and Ui (41). Proportional increases in cAMP levels and insulin secretion have been reported in response to increasing concentrations of glucose and/or glucagon in the presence or absence of IBMX. It has been suggested that increased insulin secretion during these short-term stimulations might be accounted for solely in terms of enhanced production of cAMP, regardless of the mechanism used to generate the cAMP. It was similarly implied that the intracellular processes distal to the production of cAMP were unaltered by these short-term treatments. Our results extend this proposal to a physiological condition in which islet function has been greatly enhanced by a long-term adaptation to lactogens.

Although our study demonstrated that there are increased levels of cAMP in PRL-treated islets compared with control islets at the same glucose concentrations, the mechanism by which this occurs is not completely understood. The simplest explanation would be an increase in the production of

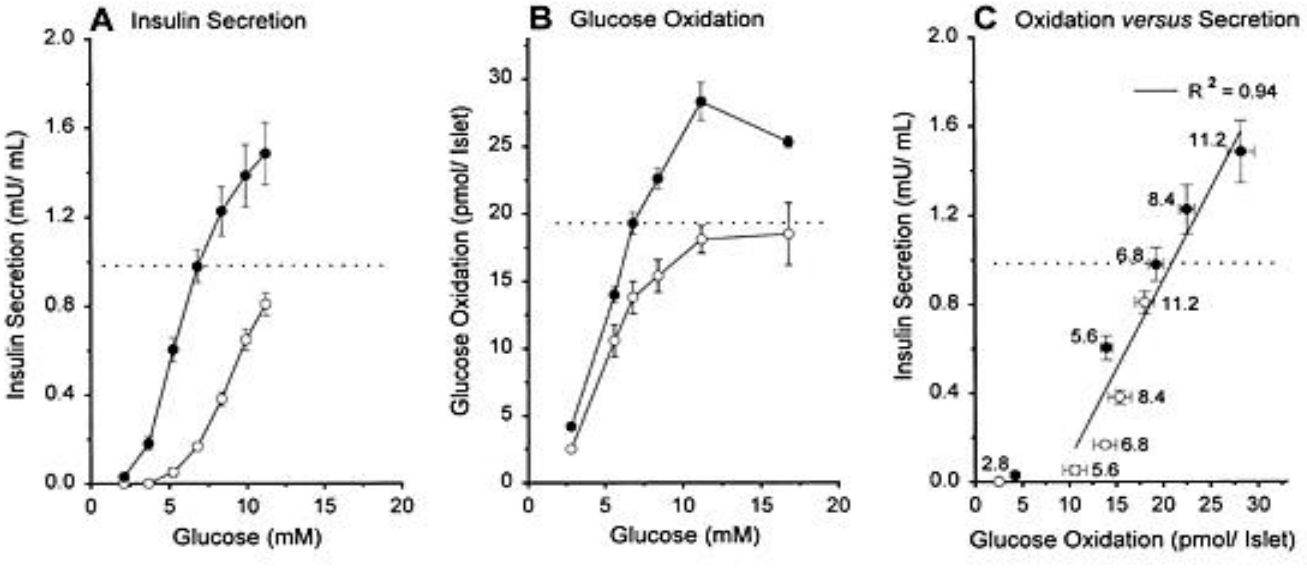

FIG. 7. Effect of PRL on insulin secretion (*A*) and glucose oxidation (*B*) in response to increasing glucose stimulation. Insulin secretion in response to a glucose gradient was measured from islets treated 4 days with PRL (●) and controls (○) in a previous study (14); glucose oxidation rates in response to increasing glucose concentrations were measured from islets treated 5 days with PRL (●) and controls (○) in a previous study (14). Both secretion and glucose oxidation from PRL-treated islets in response to a slightly stimulatory (6.8 mmol/l) glucose concentration (- - -) were higher than those from control islets in response to a high (16.8 mmol/l) glucose concentration. *C*: Correlation between insulin secretion and glucose oxidation from PRL-treated (●) and control (○) islets in response to increasing glucose concentrations from 2.8 to 11.2 mmol/l glucose. The increase in secretion was proportional to the increase in glucose oxidation above the threshold for glucose-stimulated insulin secretion, as visualized by the linear relationship (r^2 = 0.94), regardless of whether the data were from PRL-treated or control islets.

cAMP by adenylyl cyclase or a decrease in its breakdown by cyclic nucleotide phosphodiesterases. The elevated levels of cAMP in the PRL-treated islets in this study most likely reflected an increase in the production of cAMP because this change was observed in the presence of the phosphodiesterase inhibitor IBMX. In islets exposed in vivo to placental lactogens during pregnancy, an increase in adenylyl cyclase and protein kinase A activities with no change in phosphodiesterase activity was observed with islet extracts (5,11,40). However, it cannot be determined from these measurements in extracts whether the increase in adenylyl cyclase was directly responsible for the increase in cAMP metabolism in intact islets or was an indirect consequence of the increased islet function during pregnancy.

A more likely explanation is that the enhanced generation of cAMP in the PRL-treated islets arose from the increased generation of coupling factors derived from the metabolism of glucose. The best understood of these coupling factors is ATP, which blocks ATP-sensitive K^+ channels and leads to the depolarization of the β-cell membrane. This results in an increase in Ca^{2+} influx through voltage-dependent Ca^{2+} channels and the stimulation of insulin release (42,43). The Ca^{2+}-dependence of the glucose-stimulated increase in cAMP supports the proposal that the activation of adenylyl cyclase occurs through a Ca^{2+}/calmodulin complex (24,25). This suggests that increased generation of cAMP in PRL-treated islets compared with control islets at the same glucose concentration is the result of an increased rate of glucose metabolism and the generation of coupling factors that subsequently increase the intracellular Ca^{2+} concentration

The major rate-limiting step in the β-cell glycolytic pathway is the phosphorylation of glucose by the hexokinase isoenzyme, glucokinase (45). We have previously shown that PRL-treated islets have increased rates of glucose oxidation and utilization and a corresponding increase in glucokinase expression and activity (3). Similar to the pattern of changes in cAMP observed in the present study, the rate of glucose oxidation and insulin secretion at the slightly stimulatory glucose concentration (6.8 mmol/l) in PRL-treated islets was higher than that observed with the high glucose concentration (16.8 mmol/l) from control islets (Fig. 7*A* and *B*). This observation led us to examine the correlation between the rate of glucose oxidation and insulin secretion at various glucose concentrations in these islets (Fig. 7*C*). Similar to the relationship between cAMP and secretion, a linear relationship (r^2 = 0.94) was again observed for glucose-stimulated insulin secretion at glucose concentrations above the threshold, regardless of whether the data were from PRL-treated or control islets. This implies that the amount of coupling factors generated from a specific rate of glucose oxidation was unchanged during the adaptation of islets to PRL. This would suggest that the increased insulin secretion from PRL-treated islets can be accounted for solely in terms of the increased rate of glucose oxidation under these conditions. It can be further speculated that the increased metabolism of glucose in PRL-treated islets may be primarily the result of the increases in glucokinase expression and activity (3). To exclude the possibility that there is increased generation of coupling factors for a specific rate of glucose metabolism, we examined the correlation between the rate of glucose oxidation and the level of cAMP in PRL-treated and control islets (Fig. 8). The linear relationship (r^2 = 0.93) observed for values from both groups of islets implies that PRL treatment did not have a specific effect on either the production of coupling factors generated from glucose metabolism or the levels of cAMP generated in response to these coupling factors.

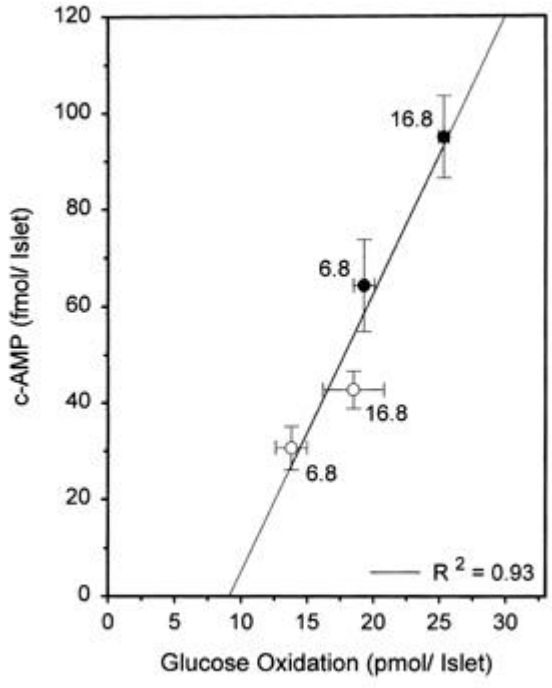

FIG. 8. Correlation between the rate of glucose oxidation (data from Fig. 7*B*) and the level of cAMP (data from Fig. 6*B*) in PRL-treated (●) and control (○) islets in response to an acute stimulation with a suprathreshold (6.8 mmol/l) or high (16.8 mmol/l) glucose concentration. The increase in cAMP concentration was proportional to the increase in glucose oxidation, as visualized by the linear relationship (r^2 = 0.93), regardless of whether the data were from PRL-treated or control islets.

Further examination of Figs. 6 and 7 shows that the threshold of glucose-stimulated insulin secretion (~5.6 mmol/l glucose in control rat islets) correlates to a rate of glucose oxidation of ~10 pmol/islet and a cAMP concentration of ~20 fmol/islet. This dependence of normal glucose-stimulated insulin secretion on a minimum rate of glucose oxidation is widely recognized; it has been demonstrated in fasted rats, which have a higher glucose-stimulation threshold and reduced insulin secretion, and in glucose-infused rats, which have a lower threshold and increased insulin secretion (12,46,47). Further support of this requirement for a minimum rate of glucose oxidation comes from the well-known inability of cAMP to potentiate insulin secretion at glucose concentrations below the glucose-stimulation threshold. However, the dependence of normal glucose-stimulated insulin secretion on a minimum concentration of cAMP is not as well appreciated. Similar to glucose-infused islets, we have previously shown that PRL-treated islets have a lowered glucose-stimulation threshold (12). Figure 7*B* shows that this lowered threshold in PRL-treated islets (~3 mmol/l) corresponds with a ~10 pmol/islet rate of glucose oxidation and a cAMP concentration of ~20 fmol/islet. This is the same rate of oxidation and cAMP generation observed at the glucose-stimulation threshold in control islets. Figure 6*C* shows that the minimum cAMP concentration required for secretion in PRL-treated and control islets occurred at ~20 fmol/islet. That the glucose-stimulation threshold in PRL-treated and control islets occurred at the minimum required cAMP concentration (which is generated by the same rate of glucose metabolism) demonstrates the dependence of insulin secretion on a minimal concentration of cAMP. Furthermore, this implies that β-cells with lower or higher cAMP levels will have a corresponding decrease or increase in insulin secretion at stimulatory glucose concentrations, respectively. This effect has been demonstrated in isolated β-cells that have a 50% reduction in cAMP compared with intact islets and a relatively poor response to glucose stimulation that is dramatically enhanced by agents that increase cAMP (14). Furthermore, we have shown that increasing islet cAMP with forskolin or glucagon results in the lowering of the stimulation threshold and an increase in glucose-stimulated insulin secretion (12). The correlation between these observations and our analysis of the data supports the idea that PRL treatment does not have a direct specific effect on the production of cAMP in islets, but rather that the production increases in response to the increased rate of glucose metabolism.

The observation that there were increased cAMP levels in the PRL-treated islets compared with control islets at the same glucose concentration can be misleading. For example, one might conclude that PRL has specific effects on cAMP metabolism or other distal events in the secretion pathway. However, PRL treatment also resulted in increased glucose metabolism at physiological glucose concentrations. Therefore any study intending to examine the PRL regulation of factors involved in stimulus-secretion coupling pathways will find differences between glucose oxidation and insulin secretion. Thus to demonstrate a specific lactogen treatment effect, it is necessary to make comparisons with islets at similar rates of insulin release, not at the same glucose concentration.

The findings of the present study raise the interesting question of whether the increases in glucokinase activity induced by PRL treatment are sufficient to explain the upregulation of insulin secretion. This is quite possible based on the analysis presented in this study. Although the increase in glucokinase may be the primary event, it is important to recognize that the subsequent increase in the recognition of glucose will also cause many other secondary events to occur in islets. For example, INS-1 cells with varying rates of glucokinase expression have corresponding changes in other factors found in the glycolytic pathway, such as the activity of L-pyruvate kinase (48). It is important that the increases in glucokinase alone were not sufficient to fully explain the changes in insulin secretion; that is, a 100% increase in glucokinase activity was needed to increase insulin secretion. In contrast, our previous studies demonstrated that a 30% increase in glucokinase was sufficient to result in the enhanced insulin secretion observed in PRL-treated islets and during pregnancy (3). This finding further demonstrates the need for additional changes in islets secondary to the increase in glucose metabolism. It remains to be determined which additional changes occur and whether these are directly regulated by PRL or are a consequence of the increase in glucose metabolism.

Our studies of upregulation of glucokinase and secretion also suggest that the long-term adaptation of islets in response to PRL is critically dependent on the presence of stimulatory concentrations of glucose. This type of glucose dependence on insulin secretion has also been observed both in vivo with dietary-restricted pregnant rats (12,46) and in vitro with islets cultured at lower glucose concentrations (49). Further experiments are needed to understand better the requirements on glucose metabolism and the sequence of

the events involved in the adaptation of islets to PRL. Understanding these processes is of importance not only to pregnancy, but also to other conditions in which there are long-term upregulatory changes in insulin secretion.

In summary, our results show that cAMP concentrations are elevated as islets adapt to an increased need for insulin secretion during pregnancy. We propose that PRL treatment does not have a direct specific effect on the production of cAMP in islets, but rather that its effect occurs in response to the increased rate of glucose metabolism. The increase in glucose metabolism and cAMP production results in the enhancement of insulin secretion under normoglycemic conditions and is the major component of the adaptation of islets to pregnancy. The results also demonstrate that the same changes that occur in islets during pregnancy can be induced by PRL treatment in vitro. This provides further evidence that the long-term adaptive changes that occur under the normoglycemic conditions of pregnancy are mediated by lactogen-regulated events.

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