Original Article

Peroxisome Proliferator–Activated Receptor-α and Glucocorticoids Interactively Regulate Insulin Secretion During Pregnancy

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We evaluated the impact of peroxisome proliferator–activated receptor (PPAR)α activation and dexamethasone treatment on islet adaptations to the distinct metabolic challenges of fasting and pregnancy, situations where lipid handling is modified to conserve glucose. PPARα activation (24 h) in vivo did not affect glucose-stimulated insulin secretion (GSIS) in nonpregnant female rats in the fasted state, although fasting suppressed GSIS. Dexamethasone treatment (5 days) of nonpregnant rats lowered the glucose threshold and augmented GSIS at high glucose; the former effect was selectively opposed by PPARα activation. Pregnancy-induced changes in GSIS were opposed by PPARα activation at day 19 of pregnancy. Dexamethasone treatment from day 14 to 19 of pregnancy did not modify the GSIS profile of perifused islets from 19-day pregnant rats but rendered the islet GSIS profile refractory to PPARα activation. During sustained hyperglycemia in vivo, dexamethasone treatment augmented GSIS in nonpregnant rats but limited further modification of GSIS by pregnancy. We propose that the effect of PPARα activation to oppose lowering of the glucose threshold for GSIS by glucocorticoids is important as part of the fasting adaptation, and modulation of the islet GSIS profile by glucocorticoids toward term facilitates the transition of maternal islet function from the metabolic demands of pregnancy to those imposed after parturition. *Diabetes* 55:3501–3508, 2006

Peroxisome proliferator–activated receptor (PPAR)α is a transcription factor upregulating expression of genes involved in lipid catabolism (1), is critical to adaptations of glucose handling to fasting (2), and is a major determinant of fatty acid oxidation in rodent islets and INS cells (3–6). Since despite hypoglycemia, PPARα-null mice exhibit hyperinsulinemia on fasting (5,7), islet PPARα expression is increased on fasting (7), and islets from PPARα-null mice show enhanced glucose-stimulated insulin secretion (GSIS) after starvation (5), it is proposed that PPARα signaling is required to suppress GSIS during fasting (5,7). Suppression of GSIS occurs in conjunction with suppression of irreversible glucose disposal via oxidation, an event linked to increased expression of the PPARα target gene pyruvate dehydrogenase kinase (PDK)4 (8–12, rev. in 13) and enhanced expression of genes related to lipid catabolism (2) in a range of tissues. Enhanced PDK4 expression and activity suppress glucose oxidation, allowing increased anaplerotic entry of pyruvate into the tricarboxylic acid cycle (rev. in 13). As mitochondrial anaplerotic products are important for GSIS (rev. in 14,15), potentiated GSIS following culture with the PPARα agonist WY14,643 by adenoviral transduction with PPARα plus its heterodimerization partner, the retinoid X receptor, may be explained by PPARα-stimulated PDK4 expression and augmented anaplerosis (6,11). However, PPARα activation could, under some conditions, promote exhaustion of a lipid-derived molecule important for optimal GSIS, thereby blunting GSIS (5). PPARα activation reverses insulin resistance (and thus the requirement for islet compensation) induced by high–saturated fat diets (16,17). The increase in lipid delivery associated with high–saturated fat diets leads to the accumulation of excess lipids ectopically in muscle, liver, and the pancreatic islet that affects metabolic signaling (rev. in 18). Such accumulated lipid-derived compounds can be cleared through PPARα activation (18).

Excess endogenous (19,20) and exogenous (21,22) glucocorticoids impair insulin sensitivity. Signaling via PPARα is essential for the induction of hepatic insulin resistance by the synthetic glucocorticoid dexamethasone (23). Glucocorticoids induce PDK4 expression in a rat hepatoma cell line, an effect opposed by insulin (24). In vivo, dexamethasone treatment of rats during the last third of pregnancy modifies regulation of maternal glucose metabolism by insulin (25). Dexamethasone also counteracts the effect of placental lactogens and prolactin to enhance insulin secretion by pancreatic islets in culture (26). These hormones have been proposed to mediate some of the islet adaptations to pregnancy (27–29).

Since effects of PPARα ablation are observed after fasting, where glycemia is in the low physiological range, and because islets from PPARα-null mice show increased insulin secretion at low glucose when previously exposed to high glucose in culture compared with islets from wild-type mice (30), we examined whether PPARα activation may regulate the threshold of GSIS during fasting (where GSIS is suppressed) or in pregnancy (where islet

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GSIS, glucose-stimulated insulin secretion; ΔI, incremental plasma insulin values integrated over the period of increased glucose; PDK, pyruvate dehydrogenase kinase; PPAR, peroxisome proliferator-activated receptor.

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compensation for maternal insulin resistance involves a lowered threshold for stimulation of insulin secretion by glucose, as well as enhanced insulin secretion at high glucose) (rev. in 31). In addition, since maternal glucocorticoids rise during starvation and during pregnancy toward term (rev. in 32), we evaluated whether there was interactive regulation of GSIS by PPARα and glucocorticoids. Our data suggest that PPARα is important for the islet response to the distinct metabolic challenges imposed by both fasting and pregnancy, situations that are associated with modified lipid handling to conserve glucose.

RESEARCH DESIGN AND METHODS

Kits for measurement of insulin by enzyme-linked immunosorbent assay were purchased from Mercodia (Uppsala, Sweden). Kits for determination of glucose (glucose oxidase) were purchased from Roche Diagnostics (Lewes, East Sussex, U.K.). Wako kits for the spectrophotometric determination of triglyceride levels were purchased from Alpha Labs (Eastleigh, U.K.). Dexamethasone (sodium phosphate) was obtained from David Bull Laboratories (Warwick, U.K.). The PPARα agonist WY14,643 (pirinixic acid) was purchased from Sigma (Poole, Dorset, U.K.). Human actrapid insulin was purchased from Novo Nordisk (Bagsvaerd, Denmark). Female Wistar rats (200–250 g) and mini-osmotic pumps were purchased from Charles River (Margate, Kent, U.K.). Standard pelleted rodent diet was purchased from Special Diet Services (Witham, Essex, U.K.). General laboratory reagents were from Roche Diagnostics or from Sigma.

Studies were conducted in adherence with the regulations of the U.K. Animal Scientific Procedures Act (1986). Age-matched female rats maintained on a standard light (0800–2000)-dark (2000–0800) cycle at 21 ± 2°C were individually housed. Rats were fed ad libitum or fasted for 48 h, as specified, with free access to water. Pregnant rats were time mated by the appearance of sperm plugs (day 0 of pregnancy). Dexamethasone was administered (100 μg/kg body wt per day) via a chronically implanted osmotic pump to nonpregnant female rats or pregnant rats from day 14 to day 19 of pregnancy (term = 22–23 days) (25). This procedure leads to almost total suppression of endogenous corticosterone levels (33). Sham operations were undertaken on the control groups. At the same time, rats were fitted with chronic indwelling jugular cannulae for infusion and sampling (34,35). WY14,643 was administered to nonpregnant or 18-day pregnant rats as a single injection (50 mg/kg body wt i.p.), and rats were sampled after a further 24 h (36,37). This period of exposure to the PPARα agonist is adequate to elicit markedly enhanced protein expression of the PPARα-linked gene *PDK4* in islets of nonpregnant rats (11). We selected this period of exposure to WY14,643 since previous studies show that as the period of PPARα activation increases beyond 2 days, pregnant (but not unmated) rats become refractory to its action (38). In addition, more prolonged PPARα activation suppresses fetal growth and increases circulating triglycerides, most likely as a consequence of increased adipocyte lipolysis (38). Control nonpregnant and 19-day pregnant rats were injected with vehicle.

Intravenous glucose tolerance tests. Glucose was administered as an intravenous bolus (0.5 g glucose/kg; 150 μl/100 g body wt) to conscious, unrestrained rats via the indwelling jugular cannula (34,35). Blood samples (100 μl) were withdrawn from the indwelling cannula, which was flushed with saline after the injection of glucose to remove residual glucose. Samples of whole blood (50 μl) were deproteinized [$ZnSO_4$/$Ba(OH)_2$], centrifuged (10,000*g* at 4°C), and the supernatant assayed for glucose. The remaining sample was immediately centrifuged (10,000*g* at 4°C) and plasma stored at −20°C until assayed for insulin. Insulin and glucose responses were used for calculation of the incremental plasma insulin values integrated over the 30-min period after the injection of glucose (ΔI_{0-30}).

Islet isolation and stepwise glucose perifusions. Rats were anesthetized (sodium pentobarbital; 60 mg/ml in 0.9% NaCl, 1 ml/kg body wt i.p.). Once locomotor activity had ceased, pancreases were excised and islets isolated by collagenase digestion. Insulin release from freshly isolated islets was measured in a perifusion system as described previously (36). Islets were perifused in basal medium (Krebs-Ringer buffer containing 20 mmol/l HEPES, pH 7.4, 5 mg/ml BSA, and 2 mmol/l glucose) for 60 min at a flow rate of 1 ml/min at 37°C before collection of fractions. Perifused islets were then subjected to square-wave stimulation, where the perifusate glucose concentration was raised to 8 mmol/l for 16 min and subsequently to 16 mmol/l for 16 min. After exposure to 16 mmol/l glucose, perifusate glucose was then lowered by switching back to basal medium. Fractions (2 ml) were collected at 2-min intervals and stored at −20°C before assay for glucose and insulin. To compare insulin secretory responses during islet perifusions, areas under the insulin curves were calculated for the entire period of the perifusion study (ΔI_{60-140}).

Hyperglycemic clamps. An intravenous glucose bolus was administered to conscious, unrestrained postabsorptive rats via an indwelling cannula, and 25% glucose solution was subsequently infused to maintain blood glucose concentrations at a target concentration of ~10 mmol/l. Blood samples were obtained before the glucose bolus and at 5-min intervals thereafter for 40 min. Samples of whole blood (50 μl) were treated as described for the intravenous glucose tolerance tests. Insulin responses were used for calculation of the incremental plasma insulin values integrated over the period of glucose infusion (ΔI_{0-40}).

Insulin clearance. A constant infusion of human actrapid insulin (4 mU · kg^{-1} · min^{-1}) was given for 2 h. Exogenous glucose infusion was initiated at 1 min after the start of insulin infusion. Blood was sampled from the right jugular vein at 5-min intervals. Blood glucose concentrations during the clamp were determined using a glucose analyzer (YSI, Yellow Springs, OH). The exogenous glucose infusion rate was adjusted to maintain glycemia. Plasma insulin concentrations reached steady state by 90 min after initiating insulin infusion; thereafter, three blood samples (0.1 ml) were obtained at 15-min intervals for measurements of plasma insulin concentrations. Insulin clearance rates were calculated by dividing the rate of insulin infusion by the steady-state insulin concentration during euglycemic hyperinsulinemia.

Statistical analyses. Results are means ± SE. Numbers of litters, rats, or islet preparations are in parentheses. Statistical analysis was by ANOVA, followed by Fisher's post hoc tests for individual comparisons or Student's *t* test as appropriate (Statview; Abacus Concepts, Berkeley, CA). A P value <0.05 was considered statistically significant.

RESULTS

PPARα activation has little effect on GSIS in the fasted state with nonpregnant female rats. We have previously demonstrated that PPARα activation in postabsorptive rats (6 h after food withdrawal) does not affect GSIS following intravenous glucose challenge in vivo or during stepwise glucose islet perifusions in vitro (36). We therefore examined GSIS after fasting (48 h), which causes increased islet PPARα signaling, indicated by enhanced islet expression of PDK4 (11) and other PPARα target genes (5). GSIS was substantially suppressed by 48 h fasting (fast group), demonstrated by a 60% decline ($P <$ 0.001) in total suprabasal 30-min area under the insulin curve (ΔI_{0-30}), following an intravenous glucose tolerance test in vivo (control 265 ± 33 vs. fast 105 ± 12 μU · ml^{-1} · min^{-1}), and a 73% decline ($P < 0.01$) in the areas under the insulin curve for GSIS with perifused islets ex vivo (ΔI_{60-140}) compared with the fed group (control 922 ± 127 vs. fast 248 ± 90 μU · ml^{-1} · min^{-1}) (Fig. 1*A*). Administration of the PPARα agonist WY14,643 after 24 h fasting, at a dose and over the time scale previously shown to increase PDK4 expression in pancreatic islets and liver (8,11), with sampling after 24 h (fast+WY group) had no further significant effect on GSIS in vivo (a ΔI_{0-30} value of 114 ± 19 μU · ml^{-1} · min^{-1}). Areas under the insulin curves (ΔI_{60-140} values) for ex vivo islet perifusions were similarly not significantly affected by WY14,643 treatment during fasting (fast+WY 180 ± 18 μU · ml^{-1} · min^{-1}) (Fig. 1*A*).

Dexamethasone treatment of nonpregnant rats lowers the glucose threshold for GSIS and augments GSIS at high glucose in islet perifusions. Glucocorticoids impair insulin sensitivity. In addition, signaling via PPARα is required for induction of hepatic insulin resistance by dexamethasone (23). Induction of PDK4 expression by elevated levels of glucocorticoids may be the primary cause of the increased PDK activity occurring during fasting (39). Increased glucocorticoids may therefore also be permissive for effects of PPARα activation to suppress GSIS on fasting. To evaluate this, we examined the effects of glucocorticoid and WY14,643 treatment alone, and in combination, on GSIS. Dexamethasone treat-

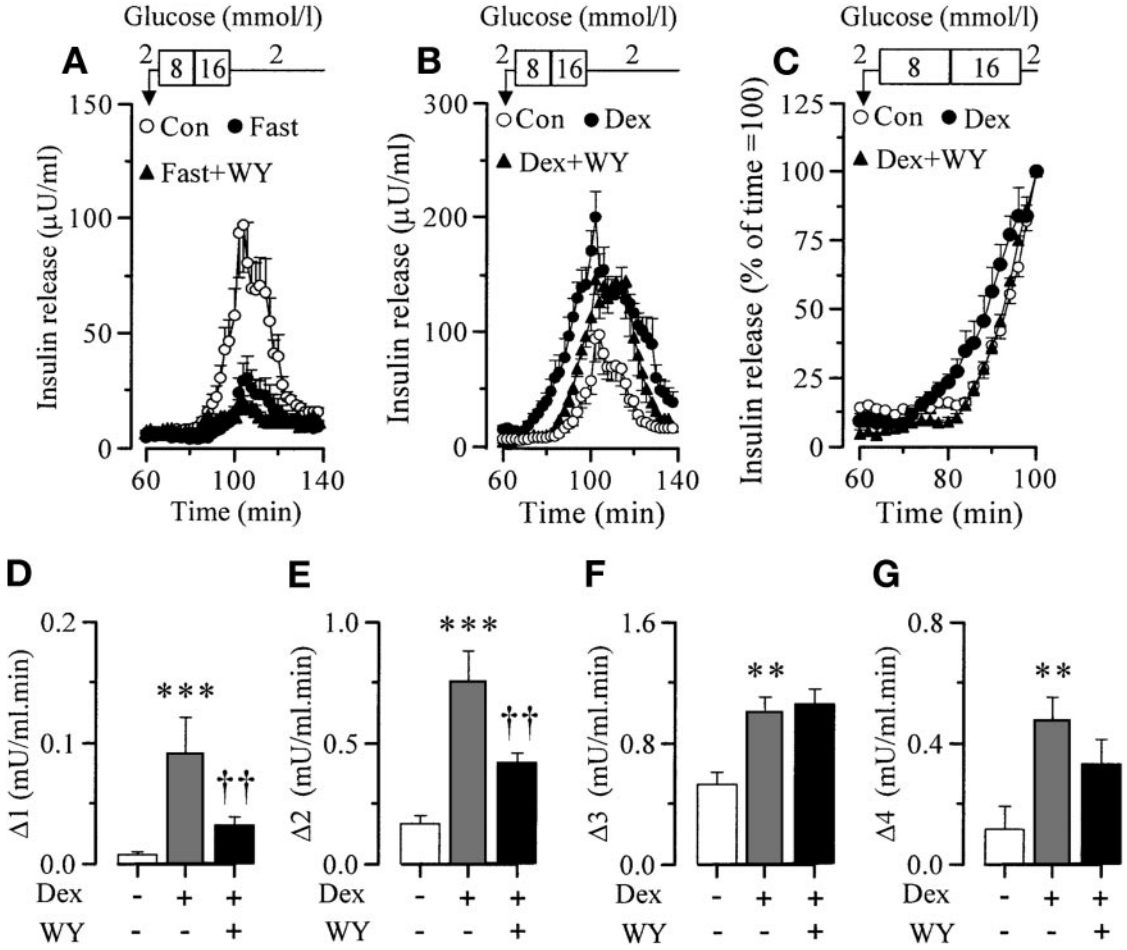

FIG. 1. Starvation and dexamethasone (Dex) treatment of nonpregnant rats modify GSIS in islet perifusions. Islets were harvested from nonpregnant ad libitum–fed rats (Con), 48-h fasted rats (Fast), 48-h fasted rats treated with WY14,643 (50 mg/kg body wt; 24 h exposure) (Fast+WY), nonpregnant ad libitum–fed rats treated with dexamethasone (100 μg/kg body wt per day; 5 days) (Dex), or nonpregnant ad libitum–fed rats treated with dexamethasone plus WY14,643 (24 h exposure, day 4–5 of dexamethasone treatment) (Dex+WY group). Islets were perifused (2 mmol/l glucose for 60 min; flow rate 1 ml/min) before being subjected to square-wave stimulation by raising the perifusate glucose concentration to 8 mmol/l (16 min) and subsequently to 16 mmol/l (16 min). After exposure to 16 mmol/l glucose, perifusate glucose was then lowered by switching back to basal medium. Fractions (2 ml) collected at 2-min intervals were assayed for insulin and glucose. *A*: Patterns of GSIS by perifused islets isolated from nonpregnant control rats (○), 48-h starved rats (●), and 48-h starved rats treated with WY14,643 (▲). *B*: Patterns of GSIS by perifused islets isolated from nonpregnant control rats (○), Dex rats (●), and Dex+WY rats (▲). *C*: Data for the control, Dex, and Dex+WY groups are expressed relative to perfusate insulin concentrations at 100 min (time = 100). *D–G*: Areas under the insulin curves for the control, Dex, and Dex+WY groups were calculated for discrete 16-min periods during perifusion in which the perifusate glucose concentration varied between 2 and 8 mmol/l and between 8 and 16 mmol/l (Δ1 and Δ2, respectively), the immediate poststimulation transition (Δ3), and the longer-term poststimulation period (Δ4). Data are means ± SE for 10 control rats, 5 fast rats, 5 fast+WY rats, 5 Dex rats, and 7 Dex+WY rats. Statistically significant effects of dexamethasone treatment are indicated by ***$P < 0.001$ and **$P < 0.01$. Statistically significant effects of WY14,643 treatment are indicated by ††$P < 0.01$.

ment suppressed food intake in nonpregnant rats by a mean 33% ($P < 0.01$) and decreased body weight on days 1 (7%; $P < 0.05$), 2 (8%; $P < 0.05$), 3 (10%; $P < 0.05$), 4 (13%; $P < 0.001$), and 5 (18%; $P < 0.001$) of treatment. Despite decreased food intake, GSIS by perifused islets isolated from nonpregnant dexamethasone-treated rats (Dex group) was higher than by perifused control islets (Fig. 1*B*). Thus, dexamethasone treatment of female rats does not elicit stable changes in islet function that mimic those of starvation but instead increases the efficacy of glucose stimulation of insulin secretion at high glucose and, as shown in Fig. 1*C*, causes a leftward shift in the glucose dose-response relationship. A lowered glucose threshold has been observed previously with islets prepared from male rats treated with dexamethasone (40).

PPARα activation specifically opposes lowering of the glucose threshold by dexamethasone with islets from nonpregnant rats. We examined effects of 24 h in vivo exposure to the PPARα agonist WY14,643 in perifused islets from dexamethasone-treated (nonpregnant) female rats (Dex+WY group; Fig. 1*B*). PPARα activation failed to modify glucose responsiveness of GSIS but modulated glucose sensitivity. To quantify differences in the glucose threshold for GSIS between perifused islets from control, Dex, and Dex+WY groups, areas under the insulin curves were calculated for 16-min periods, during which perifusate glucose was rising: Δ1, during which perifusate glucose varied between 2 and 8 mmol/l, and Δ2, during which the perifusate glucose concentration varied between 8 and 16 mmol/l. Similarly, areas under the insulin curves were calculated for equivalent 16-min periods of perifusion after switching to basal glucose: Δ3, the immediate poststimulation transition after removal of the 16 mmol/l glucose stimulus and restoration of basal glucose, and Δ4, the longer-term poststimulation period. Data are shown in Fig. 1*D–G*. Dexamethasone treatment of nonpregnant rats increased GSIS over the entire range of glucose concentrations, as shown by significantly higher values of Δ1, Δ2, Δ3,

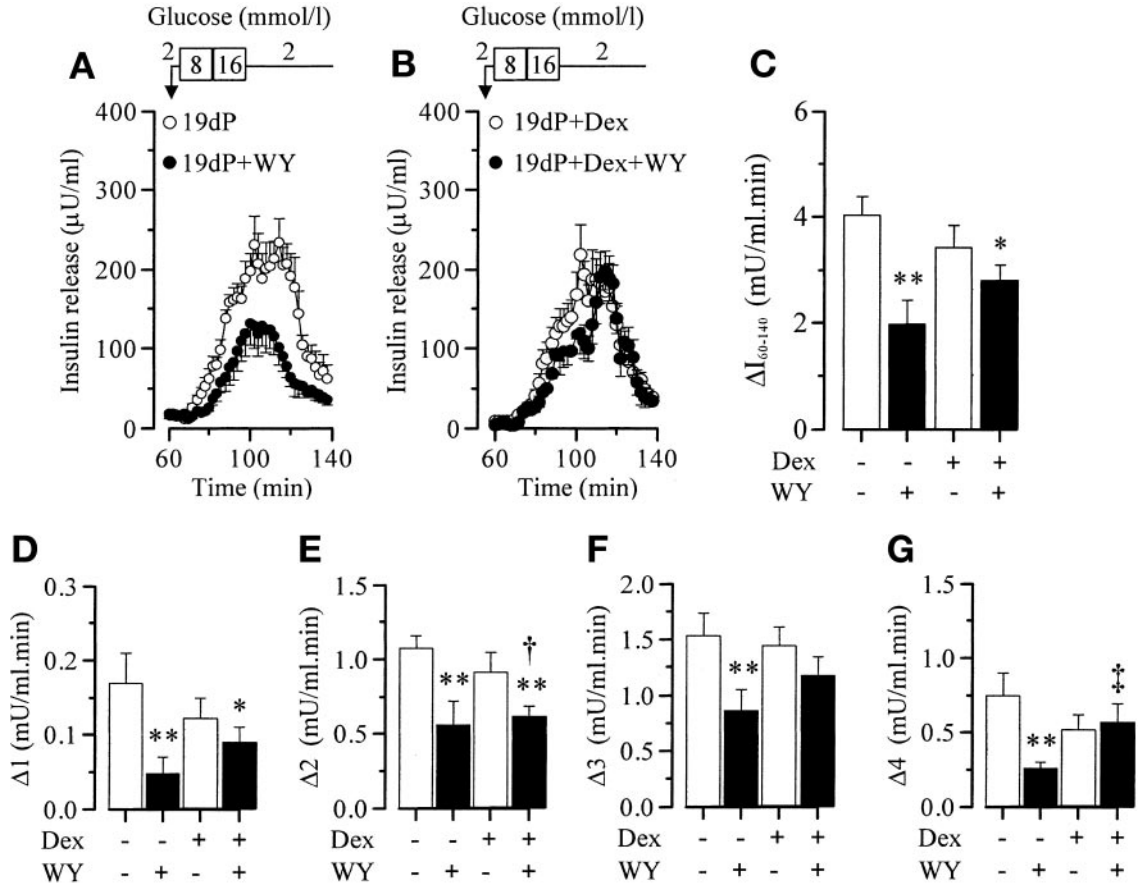

FIG. 2. The pattern of response of GSIS with perifused islets from 19-day pregnant rats, with or without dexamethasone (Dex) treatment, to antecedent PPARα activation in vivo. Dexamethasone was administered subcutaneously (100 μg/kg body wt per day) for 5 days from day 14 of pregnancy (19dP+Dex group). WY14,643 (50 mg/kg body wt i.p.) was administered to pregnant rats with (19dP+Dex+WY group) or without (19dP+WY) dexamethasone treatment on day 18 of pregnancy and rats sampled at day 19 of pregnancy. *A*: Data for perifused islets from 19-day pregnant rats (19dP; open symbols) and 19dP+WY rats (closed symbols). *B*: Data for perifused islets from 19dP+Dex and 19dP+Dex+WY rats (▲). *C*: Areas under the insulin curves calculated for the period over which perifusate glucose exceeded basal (ΔI_{60-140}). *D–G*: Δ1, Δ2, Δ3, and Δ4 values. Data are means ± SE for six 19dP, five 19dP+WY, five 19dP+Dex, and five 19dP+Dex+WY rats. Statistically significant differences from the untreated 19-day pregnant group are indicated by *$P < 0.05$ and **$P < 0.01$. Statistically significant differences from the dexamethasone-treated 19-day pregnant group are indicated by †$P < 0.05$. Statistically significant effects of dexamethasone treatment are indicated by ‡$P < 0.05$.

and Δ4. Contrasting with its failure to significantly affect GSIS at low glucose concentrations with perifused islets from nonpregnant rats (36), antecedent PPARα activation lowered Δ1 and Δ2 values for GSIS in the dexamethasone-treated nonpregnant rats (Fig. 1*D* and *E*). Antecedent PPARα activation did not affect Δ3 values in the dexamethasone-treated group (Fig. 1*F*). The overall pattern of the responses of Δ4 values to antecedent PPARα activation (Fig. 1*G*) qualitatively resemble that of Δ2, indicating that GSIS in dexamethasone-treated rats regains susceptibility to suppression by PPARα activation as extracellular glucose concentrations fall. PPARα activation therefore selectively opposes the leftward shift in the glucose threshold induced by dexamethasone treatment, while exhibiting a negligible effect on GSIS at high glucose concentrations.

Differential responsiveness of GSIS to antecedent PPARα activation is observed as the duration of pregnancy proceeds. Pregnant rats were treated with WY14,643 at day 18 of pregnancy, and islets were prepared at day 19 for perifusion (19dP group). The efficacy of PPARα activation during late (18–19 days) pregnancy was demonstrated by the action of WY14,643 to significantly decrease circulating triglyceride concentrations by 41% ($P < 0.05$) (19dP 1.40 ± 0.31 vs. 19dP+WY 0.82 ± 0.17 mmol/l). GSIS by perifused islets from 15-day pregnant rats is unaffected by antecedent PPARα activation in vivo (36). In marked contrast, GSIS by perifused islets from 19-day pregnant rats (Fig. 2*A*) was markedly suppressed by antecedent PPARα activation in vivo (19P+WY group). Both the glucose threshold and glucose responsiveness of GSIS were affected by PPARα activation at this stage of pregnancy, as shown by lower values for Δ1, Δ2, Δ3, and Δ4 (Fig. 2*D–G*). It can therefore be concluded that the progression of pregnancy from day 15 (mid pregnancy) to day 19 (late pregnancy) is associated with increased susceptibility of GSIS to modulation by PPARα activation.

Dexamethasone treatment in vivo does not alter the pattern of GSIS with perifused islets from 19-day pregnant rats. Since maternal glucocorticoids increase during late pregnancy to assist fetal maturation (32) and increased glucocorticoids may be permissive for effects of PPARα activation to increase PDK4 during fasting (24), we examined whether glucocorticoid treatment from days 14 to 19 of pregnancy further modified GSIS or PPARα signaling to GSIS during late pregnancy. Dexamethasone treatment (19dP+Dex group) suppressed maternal food intake on days 15 (16%; $P < 0.01$), 16 (19%; $P < 0.001$), and 17 (12%; $P < 0.05$) of pregnancy. Maternal body weight was lowered on days 17 (9%; $P < 0.01$), 18 (8%; $P < 0.05$), and

19 (11%; $P < 0.001$) of pregnancy. Fetal number and viability were not compromised (25,33,41), but mean fetal weight at day 19 of pregnancy was decreased by 33% ($P < 0.01$) (19dP 2.6 ± 0.1 g [$n = 6$] vs. 19dP+Dex 2.2 ± 0.1 g [$n = 5$]). Data for perifused islets from 19dP and 19dP+Dex groups are shown in Fig. 2*A* and *B*, respectively. Neither ΔI_{60-140} values (Fig. 2*C*) nor the glucose threshold for GSIS (as indicated by unaltered Δ1 and Δ2 values; Fig. 2*D* and *E*) were significantly modified by dexamethasone treatment from day 14 to day 19 of pregnancy.

Effects of dexamethasone on GSIS are dominant to those of pregnancy. To summarize, PPARα activation selectively opposes the leftward shift in the glucose threshold induced by dexamethasone treatment in nonpregnant rats, while exhibiting a negligible effect on GSIS at high glucose concentrations (Fig. 1). In contrast, PPARα activation opposes both the leftward shift in the glucose threshold and the increase in glucose responsiveness observed at day 19 of pregnancy (Fig. 2). The similarities between the GSIS profiles for 19dP and 19dP+Dex islets (Fig. 2) suggest that the stable modifications of islet function that result in altered GSIS, elicited by either dexamethasone treatment or late pregnancy, might be exclusive. We examined the pattern of response to PPARα activation in pregnant rats treated with dexamethasone from day 14 to 19 of pregnancy to examine whether the pattern of response more closely resembled that seen after dexamethasone treatment in nonpregnant rats or that observed at late (19 day) pregnancy (without dexamethasone treatment). Data are shown in Fig. 2. As was observed for the untreated 19-day pregnant rats, WY14,643 treatment of dexamethasone-treated pregnant rats elicited a significant 53% ($P < 0.05$) decline in circulating triglyceride concentrations, indicating the efficacy of WY14,643 treatment. WY14,643 treatment of dexamethasone-treated pregnant rats had a very limited effect on glucose responsiveness, but there was modest suppression of Δ1 and Δ2 (Fig. 2*D* and *E*). Thus, the effect of dexamethasone on glucose responsiveness appears dominant to that of pregnancy. Furthermore, the pattern of response of GSIS to PPARα activation at day 19 of pregnancy, following dexamethasone administration from day 14 to day 19 of pregnancy, is reminiscent of that observed at day 15 of pregnancy (without dexamethasone treatment [36]).

Dexamethasone treatment augments insulin secretion during hyperglycemic clamps in nonpregnant rats but not late pregnant rats. To test whether the effect of dexamethasone treatment from day 14 to day 19 of pregnancy limits islet adaptations occurring from day 15 to day 19 of pregnancy, we analyzed insulin responses during hyperglycemic clamp studies in vivo, in which glucose was infused to maintain glycemia at ~10 mmol/l for 40 min. Data for nonpregnant rats (with or without dexamethasone treatment) are shown in Fig. 3*A–C*. At similar steady-state glycemia, steady-state plasma insulin levels were 2.6-fold higher ($P < 0.05$) in the Dex group versus the control group (Fig. 3*B*), and the Dex group exhibited a 2-fold greater incremental area under the insulin curve (ΔI_{0-40}) (Fig. 3*C*). Data for 19-day pregnant rats (with or without dexamethasone treatment) are shown in Fig. 3*D–F*. Steady-state plasma insulin levels were 4.1-fold higher ($P < 0.001$) in the 19dP group compared with unmated control group (compare open symbols in Fig. 3*B* and *E*). ΔI_{0-40} was 4.7-fold higher ($P < 0.001$) in the 19dP group compared with the control group (compare open bars in Fig. 3*C* and *F*). Steady-state plasma insulin levels did not differ significantly between Dex and 19dP+Dex groups (compare closed bars in Fig. 3*C* and *F*). Comparison of the 19dP and 19dP+Dex group shows that dexamethasone treatment during pregnancy decreases ΔI_{0-40} by 51% ($P < 0.05$) and steady-state plasma insulin levels by 57% ($P < 0.05$) to values not different from those of the Dex group. Thus, dexamethasone treatment and pregnancy do not exert additive effects on GSIS in vivo. We infused insulin at a fixed rate (4 mU · kg^{-1} · min^{-1}), while maintaining glycemia, to determine whether decreased insulin levels in the 19dP+Dex group, compared with the 19dP group, reflected increased insulin clearance. The coefficients of variance for glycemia were <15%, and steady-state glycemia attained during euglycemic hyperinsulinemia were similar in the control and dexamethasone-treated groups of pregnant rats (data not shown). At similar steady-state glycemia, steady-state plasma insulin concentrations during insulin infusion did not differ significantly between control and dexamethasone-treated dams (19dP 69 ± 7 μU/ml [$n = 10$] vs. 19dP+Dex 67 ± 5 μU/ml [$n = 6$]). Hence, insulin clearance is unaffected by dexamethasone treatment during pregnancy (19dP 22.3 ± 2.2 ml/min [$n = 10$] vs. 19dP+Dex 18.6 ± 1.1 ml/min [$n = 6$]).

DISCUSSION

Our data demonstrate a marked effect of dexamethasone treatment to modify the characteristics of GSIS with perifused islets from nonpregnant rats, with a lowering of the glucose threshold for GSIS and an increased response of GSIS to glucose at high concentrations; the latter reproducing the enhanced GSIS observed in nonpregnant rats treated with dexamethasone during hyperglycemic clamps in vivo. Although this at first sight appears at variance with a role for glucocorticoids in facilitating suppression of GSIS during fasting, these data are consistent with adaptations of the islet to the development of insulin resistance in response to dexamethasone administration, allowing greater insulin secretion to attempt to compensate for impaired insulin action. Furthermore, our data also demonstrate that the lowering of the glucose threshold evoked by dexamethasone in unmated rats is markedly and selectively opposed by PPARα activation. Fasting increases circulating glucocorticoid levels, and increased glucocorticoid action is an integral component of the metabolic response to fasting. On the basis of the present data, we suggest that exposure to glucocorticoids is permissive for the islet response to PPARα activation during the transition from the fed to the fasted state and that the reversal of glucocorticoid-induced lowering of the glucose threshold for GSIS by PPARα activation is important to ensure that during fasting the islet does not perceive elevated glucocorticoid levels as a signal for compensatory insulin secretion. The lowering of maximal responsiveness of GSIS to glucose, which we suggest may be occurring, in response to increased glucocorticoid concentrations during fasting does not appear to be mediated through increased PPARα-linked functions, as PPARα activation exhibited a negligible effect on GSIS at high glucose concentrations in the dexamethasone-treated rats (nonpregnant group). This may explain why the lowered capacity for GSIS observed after fasting can be "rescued" by in vivo lipid administration. Lipid administration has been proposed to replenish a lipid intermediate important for GSIS (42,43). However, our data showing a

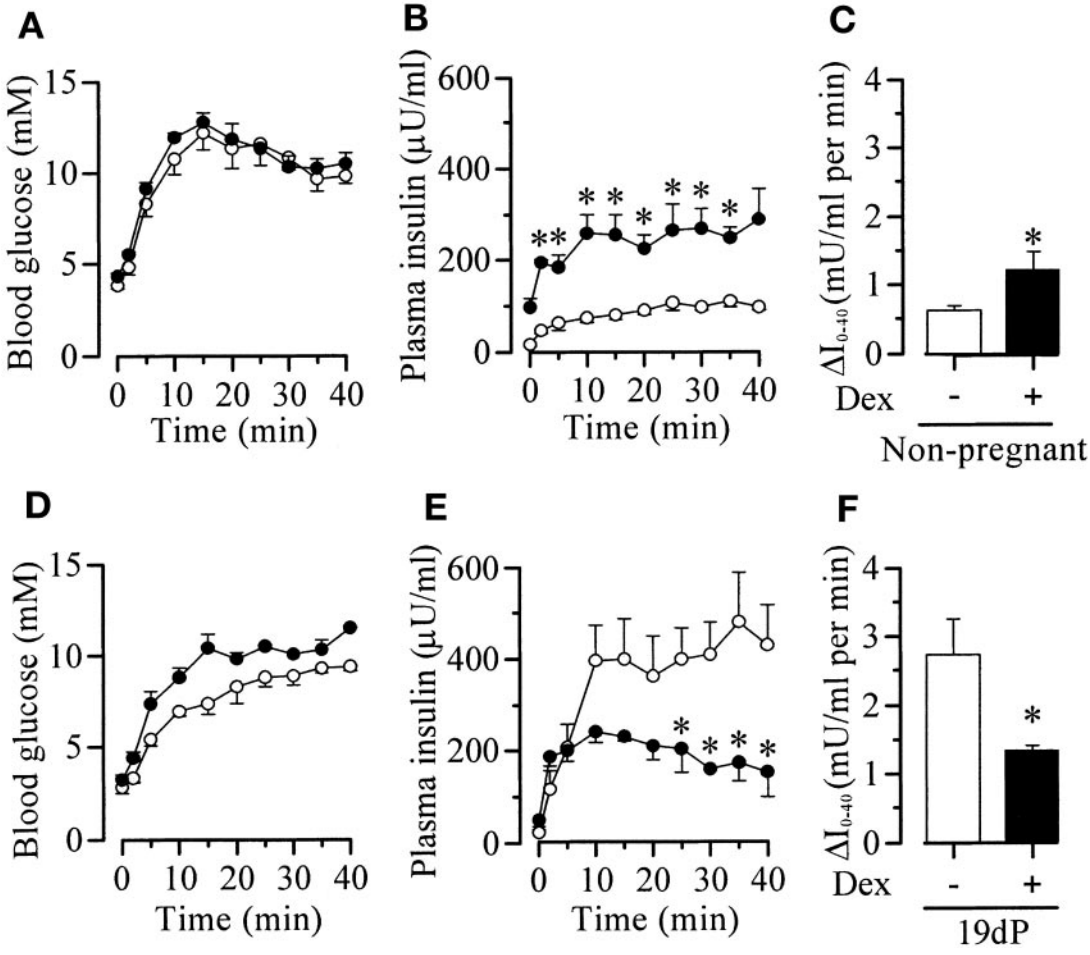

FIG. 3. Dexamethasone (Dex) treatment augments insulin secretion during hyperglycemic clamp in vivo in nonpregnant but not in late (19 day)-pregnant rats. Blood samples were withdrawn from nonpregnant control rats (open symbols/bars) or dexamethasone-treated nonpregnant rats (Dex group; closed symbols/bars) (*A–C*) or untreated 19-day pregnant rats (19dP; open symbols/bars) or dexamethasone-treated 19-day pregnant rats (19dP+Dex; closed symbols/bars) (*D–F*) at intervals during sustained intravenous glucose infusion for measurement of blood glucose (*A* and *D*) and plasma insulin concentrations (*B* and *E*). Mean steady-state blood glucose concentrations over the period of 20–40 min after initiating glucose infusion were: control 10.7 ± 0.8 mmol/l (5), Dex 10.8 ± 0.4 mmol/l (5), 19P 9.2 ± 0.3 mol/l (6), and 19P+Dex 10.5 ± 0.2 mmol/l (5). Corresponding steady-state plasma insulin concentrations were: control 101 ± 9 μU/ml (5), Dex 258 ± 41 μU/ml (5), 19P 416 ± 77 μU/ml (6), and 19P+Dex 179 ± 33 μU/ml (4). Incremental plasma insulin values integrated over the period of 0–40 min after the initiating hyperglycemia (ΔI_{0-40}) are also shown (*C* and *F*). Data are means ± SE for five control, five Dex, six 19P, or five 19P+Dex rats. Statistically significant effects of dexamethasone treatment are indicated by *$P < 0.05$. Statistically significant effects of pregnancy are reported in the text.

lack of an effect of pharmacological PPARα activation during fasting is most likely explained by PPARα being already fully activated by endogenous ligands.

Pregnancy is a lifestyle factor that leads to adaptive changes in pancreatic β-cell function, including increased GSIS and a reduction in the glucose-stimulated threshold for insulin secretion. The latter is thought to be the primary mechanism by which the pancreatic β-cells can release significantly more insulin at low blood glucose concentrations, resulting in an altered set point for insulin secretion and action during late pregnancy. We have previously observed that PPARα activation fails to impact on the characteristics of GSIS at day 15 of pregnancy (36). In contrast, in the present study during late pregnancy (day 19; term = 22–23 days), PPARα activation both increased the glucose threshold for GSIS and lowered glucose responsiveness. There is therefore a shift in the functional characteristics of GSIS, with respect to PPARα activation, as pregnancy proceeds toward term. Importantly, PPARα activation later in pregnancy leads to a pattern of GSIS at day 19 of pregnancy (i.e., late gestation, seen in the present study) reminiscent of that seen earlier at day 15 of pregnancy (36). This suggests that the enhancement of GSIS observed at day 19 of pregnancy compared with day 15 of pregnancy is opposed by increased PPARα signaling. It is likely that the effects of PPARα activation on GSIS in late pregnancy observed in the present studies reflect changes in the acute stimulation of the release of stored insulin from docked granules by the ATP-sensitive K^+ channel–dependent mechanism; however, we were unable to detect a clear first- and second-phase secretion under either the conditions of the rat islet perifusions in vitro or in vivo during long-term hyperglycemic clamp studies.

Glucocorticoid concentrations increase during late pregnancy to assist maturation of fetal tissue function (32). We aimed to establish whether increased exposure to glucocorticoids might contribute to the mechanism of this phenotypic shift in the characteristics of GSIS. Glucocorticoid treatment from day 14 to day 19 of pregnancy in the absence of PPARα activation appeared to exert only a minimal effect on the characteristic pattern of the GSIS profile ex vivo with perifused islets from late pregnant rats; a lowered glucose threshold and increased glucose

responsiveness continued to be observed. However, following antecedent treatment with exogenous glucocorticoids, the glucose responsiveness of perifused islets of late-pregnant rats became refractory to suppression by PPARα activation, as is the increased glucose responsiveness observed with perifused islets from nonpregnant rats treated with dexamethasone or with islets from pregnant rats at day 15 of pregnancy (36). Dexamethasone has been shown by others to exert a significant inhibitory effect on insulin secretion by islets from neonatal rats previously cultured with lactogens (26). Our experiments also demonstrated that glucocorticoid treatment suppresses GSIS in vivo during hyperglycemic clamps during late pregnancy to levels of GSIS elicited by glucocorticoid treatment of nonpregnant rats. The use of islet perifusions eliminates neuro, hormonal, and metabolic influences on GSIS. The disparity between the data obtained in vivo (suppression of GSIS) and in vitro with perifused islets (unimpaired GSIS) in the pregnant rats may reflect that dexamethasone-induced insulin resistance causes islet adaptations to augment GSIS, which are stable to islet isolation and perifusion, but that GSIS is suppressed in vivo by one or more neuro/hormonal influences that prevents the full function of these adaptations. Based on these data, we propose that increased exposure to glucocorticoids, as the duration of pregnancy proceeds beyond 19 days toward term, could have a physiological function in that islet glucose responsiveness is increasingly and selectively governed by the maternal glucocorticoid level rather than "pregnancy adaptations" introduced by placental lactogens and prolactin, which occur earlier during gestation. This may allow a smoother transition of maternal islet function from the metabolic demands imposed by pregnancy to those imposed after parturition.

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