PPARδ and its activator PGI₂ are reduced in diabetic embryopathy: involvement of PPARδ activation in lipid metabolic and signalling pathways in rat embryo early organogenesis

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Maternal diabetes significantly increases the risk of congenital malformations, and the mechanisms involved are not yet clarified. This study was designed to address peroxisome proliferator-activated receptor δ (PPARδ) involvement in diabetic embryopathy. We investigated the concentrations of PPARδ and its endogenous agonist prostaglandin (PG)I₂, as well as the effect of PPARδ activation on lipid metabolism and PGE₂ concentrations in embryos from control and streptozotocin-induced diabetic rats during early organogenesis. Embryos from diabetic rats showed decreased concentrations of PPARδ and its endogenous agonist PG₁₂ when compared with controls. In embryos from control rats, the addition of the PPARδ activators (cPGI₂ and PGA₁) increased embryonic phospholipid levels and de novo phospholipid synthesis studied using ¹⁴C-acetate as a tracer. PGE₂ formed from arachidonate released from phospholipid stores was also up-regulated by PPARδ activators. In embryos from diabetic rats, reduced phospholipid synthesis and PGE₂ content were observed, and clearly up-regulated by cPGI₂ additions to values similar to those found in control embryos. These data suggest that PPARδ may play an important role in lipid metabolic and signalling pathways during embryo organogenesis, developmental pathways that are altered in embryos from diabetic rats, possibly as a result of a reduction in levels of PPARδ and its endogenous activator PG₁₂.

Key words: diabetes in pregnancy/lipid mediators/embryo development/PPARs/prostaglandins

Introduction

An increased incidence of congenital malformations has been found in the offspring of both human and experimental diabetic pregnancies (Schwartz and Teramo, 2000; Eriksson et al., 2003). These malformations are mostly induced during early organogenesis, and several mechanisms of teratological importance are involved in their aetiology, although not completely understood (Eriksson et al., 2003; Jawerbaum and Gonzalez, 2005; Locken, 2006). In addition to hyperglycaemia, alterations in lipid metabolism affect both diabetic mothers and their developing embryos (Singh and Feigelson, 1983; Herrera and Amusquivar, 2000; Sinner et al., 2003). Lipids play an essential role in embryonic growth and development as components of the newly formed cell membranes, as oxidative fuels and also as signalling molecules (Herz and Farese, 1999). During early organogenesis, it is mainly the yolk sac membrane that allows the embryo access to lipoproteins from the maternal circulation (Jollie, 1990). This access is particularly important for the closure of the neural tube, where an enormous cellular expansion at the neural folds is critical for normal development. Accordingly, defects in endogenous lipid biosynthesis, receptor-mediated endocytosis and receptor-mediated transfer of lipids through the yolk sac, either through the effect of pharmacological agents or through genetic mutations, can lead to disrupted development (Jollie, 1990; Herz and Farese, 1999).

Our previous studies have shown that lipid synthesis of triglycerides, cholesterol, phospholipids and cholesteryl esters are reduced in embryos from diabetic rats during early organogenesis, although their levels are compensated by an increased maternal lipid transfer. An increase in triglycerides has been detected in these embryos (Sinner et al., 2003). Both cholesterol and phospholipids are crucial components of both surface and nuclear cell membranes and determine their physicochemical characteristics. In addition, cholesterol is a morphogen needed for embryo development (Porter et al., 1996), and phospholipids are the source of arachidonic acid, substrate for the synthesis of prostaglandins (PGs) (Smith et al., 1996).

PGs are oxygenated metabolites of the 20-carbon polyunsaturated fatty acid molecule arachidonic acid, which is released from membrane phospholipids by the action of phospholipases, mainly phospholipase A₂. Cyclooxygenases (COX-1 and COX-2) catalyse the conversion of arachidonic acid into PGH₂, the initial step in PG biosynthesis (Smith et al., 1996). PGH₂ is subsequently converted to one of several structurally related PGs, mainly PGE₂, PGD₂, PGF₂ and PGI₂, by the activity of specific PG synthases. PGE₂ is a lipid messenger involved in neural tube closure during early embryo organogenesis (Piddington et al., 1996). Several studies have implicated altered PGE₂ formation in diabetic embryopathy (Wiznitzer et al., 1999;
Eriksson et al., 2003; Jawerbaum and González, 2006). Indeed, PGE\textsubscript{2} content is reduced in embryos from diabetic rats and in normal embryos cultured in the presence of either diabetic or hyperglycaemic serum (Piddington et al., 1996; Jawerbaum et al., 2001; Wentzel and Eriksson, 2005). Moreover, both PGE\textsubscript{2} and arachidonic acid supplementation have been shown in vivo and in vitro to protect against diabetic malformations (Goto et al., 1992; Reece et al., 1996).

PGI\textsubscript{2} is another PG that prevents hyperglycaemia-induced embryo malformations and has been found reduced in both maternal and neonatal tissues (Stuart et al., 1981; Baker et al., 1990; White et al., 2002). Recent studies assign an important role of PGI\textsubscript{2} in implantation, because it rescues the implantation defects of COX-2 (\texttext.GetTextArrow{\text{-}}) knockout mice (Lim et al., 1999). Interestingly, PGI\textsubscript{2} is a naturally occurring agonist of peroxisome proliferator-activated receptor \(\delta\) (PPAR\(\delta\)), and through its activation, PPAR\(\delta\) has been proposed to be a critical mediator of embryo implantation (Lim and Dey, 2000).

PPAR\(\delta\) is one of three subtypes of PPARs, which are nuclear receptors that act as regulatory transcription factors, heterodimerize with retinoid X receptors and modulate gene expression of target genes containing peroxisome proliferator-responsive elements (PPREs) in response to ligand activation (Barish et al., 2006). PPARs are key regulators of adipocyte differentiation and lipid homeostasis (Desvergne et al., 2004). Their pharmacological ligands, thiazolidinediones (PPAR\(\gamma\) agonists) and fibrates (PPAR\(\alpha\) activators), are employed to ameliorate altered lipid profiles and insulin resistance in metabolic syndrome (Desvergne et al., 2004). Each PPAR subtype has particular tissue distributions, physiological functions and ligands. PPAR\(\gamma\) ligands are PGI\textsubscript{2}, PGA\textsubscript{1}, iloprost and carbaprostacyclin (cPGI\textsubscript{2}), as well as various saturated and polyunsaturated fatty acids (Forman et al., 1999). Although PPAR\(\delta\) is the least studied PPAR, it is now clear that PPAR\(\delta\) regulates skeletal muscle lipid metabolism, and thus pharmacological agonists are currently under development as promising agents to regulate lipid homeostasis in metabolic syndrome (Barish et al., 2006). Apart from lipid metabolism, PPAR\(\delta\) has also been involved in the control of cell survival and proliferation, and in wound repair (Michalik et al., 2002).

PPAR\(\delta\) is the only PPAR isoform expressed during rat early embryo organogenesis (Braisant and Wahli, 1998). Although ubiquitously expressed in the adult, expression of PPAR\(\delta\) is considerably higher in the developing neural tube during rat development (Braisant and Wahli, 1998). Nevertheless, whether PPAR\(\delta\) is involved in early embryo organogenesis, the period where most malformations, mainly neural tube defects, are induced remains largely unknown.

Given the expression of PPAR\(\delta\) during embryo organogenesis, its intimate relationship with lipid homeostasis and the alterations induced in embryo lipid metabolism by maternal diabetes, we hypothesized that PPAR\(\delta\) activators may regulate embryo lipid metabolic and signalling pathways and that alterations in these pathways may be involved in diabetic embryopathy. Therefore, we evaluated the influence of PPAR\(\delta\) activators on lipid levels, lipid synthesis and PGE\textsubscript{2} production in embryos obtained from control and diabetic rats during early organogenesis, and we measured embryonic PPAR\(\delta\) and its endogenous agonist PGI\textsubscript{2}.

### Animals, materials and methods

#### Animals

Albino Wistar rats bred in the laboratory were fed Purina rat chow ad libitum. Female rats weighing 200–230 g were made diabetic with a single i.p. injection of streptozotocin (55 mg/kg) (Sigma-Aldrich, St Louis, MO, USA) in citrate buffer (0.05 M, pH 4.5), as previously described (Jawerbaum et al., 2001). Control rats were injected with buffer only. Diabetic rat glycaemia was measured with glucostix reagent strips and a glucometer (Bayer Diagnostics, Buenos Aires, Argentina). Estrous cycles in diabetic rats were present for 2/3 weeks after streptozotocin administration. Both normal and diabetic females were mated with control male rats. Mating was confirmed by the presence of sperm in vaginal smears. When a positive pregnancy was identified, this was designated day 0.5 of gestation. The guidelines for the care and use of animals approved by the local institution were followed, according to ‘Principles of laboratory animal care’ (NIH publication No. 85–23, revised 1985).

Animals were killed by cervical dislocation on day 10.5 of pregnancy, a period corresponding to early organogenesis, and the uteri were transferred to Petri dishes with Krebs Ringer Bicarbonate (KRB) solution: 11.0 mM glucose, 145 mM Na\textsubscript{+}, 2.2 mM Ca\textsuperscript{2+}, 1.2 mM Mg\textsuperscript{2+}, 127 mM Cl\textsuperscript{−}, 25 mM HCO\textsubscript{3}\textsuperscript{−}, 1.2 mM SO\textsubscript{4}\textsuperscript{2−} and 1.2 mM PO\textsubscript{4}\textsuperscript{3−}. By the use of a stereomicroscope and microsurgical dissecting instruments, the balls of decidual tissue were removed from each uterus and gently opened to free the conceptuses. The embryos were dissected out of the yolk sacs and evaluated morphologically under a stereomicroscope. Viability was established by the presence of a beating heart. The embryos were categorized as morphologically normal or as showing either neural tube defects or other malformations. Embryonic growth was quantified by direct measurement of protein content by the Bradford method using a protein assay reagent (Bio-Rad Laboratories Inc., CA, USA) with bovine serum albumin (BSA) as standard. Embryos in resorption stages received no further analyses. Viable embryos were immediately prepared according to the following determinations.

#### Enzyme immunoassay of PGI\textsubscript{2}

PGI\textsubscript{2} was measured in control and diabetic embryos by the evaluation of PGI\textsubscript{2} stable metabolite 6-keto-PGF\textsubscript{1\alpha}, employing a commercial enzyme immunoassay kit (Cayman Chemical Co., Ann Arbor, MI, USA). Four embryos from each rat (\(n = 10–12\) rats in each experimental group) were selected at random, homogenized and sonicated in phosphate-buffered saline (PBS) and an aliquot separated for protein determination by Bradford method using a protein assay reagent (Bio-Rad Laboratories Inc.). PGs were extracted twice in absolute ethanol. The extracts were dried in a Savant (Hicksville, NY, USA) Speed-Vac concentrator and reconstituted with 200 \(\mu\)l of assay buffer provided by the commercial kit. Briefly, the kit uses a polyclonal antibody against 6-keto-PGF\textsubscript{1\alpha} to bind in a competitive manner the PG in the sample or an acetylcholinesterase molecule, which has 6-keto-PGF\textsubscript{1\alpha} covalently attached to it. After a simultaneous incubation, a p-nitrophenyl phosphate substrate is added, and the yellow colour generated is evaluated on a microplate reader at 405 nm. Results are expressed as pg/\(\mu\)g protein.

#### Western blot analysis of PPAR\(\delta\)

Seven embryos from each rat (\(n = 8\) rats in each experimental group) were selected at random for the determination of PPAR\(\delta\) protein expression by western blot. The embryos were homogenized and sonicated in 200 \(\mu\)l of ice-cold lysis buffer (pH 7.4, 20 mM Tris–HCl, 150 mM NaCl, 1% Triton X-100) containing 1% protease inhibitor cocktail and then incubated on ice for 2 h. Embryonic tissues were centrifuged at 7200 \(g\) for 10 min at 4°C and the supernatant removed. Protein concentrations were determined by Bradford method using a protein assay reagent (Bio-Rad Laboratories Inc.). Equal amounts of protein samples (50 \(\mu\)g per lane) were separated with the use of 12% sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). Proteins were then transferred onto nitrocellulose membranes, which were blocked with 1% BSA for 1.5 h and then incubated with a polyclonal rabbit IgG antibody either against PPAR\(\delta\) (1:200) (Santa Cruz Biotechnology, CA, USA) or against \(\alpha\)-actin (Sigma-Aldrich) at 4°C overnight. After washing with Tris buffer saline and Tween 0.05%, the blots were treated with horseradish peroxidase-conjugated secondary antibody for 1 h and washed several times. The specific signals were visualized using the (ECL) enhanced chemiluminescence system (Amersham Biosciences, Arlington Heights, IL, USA). The identity of PPAR\(\delta\) was established by the use of molecular weight standards and a cell lysate from rat lung epithelium as a positive control, which allows the identification of the band revealed at the expected size of 50 kDa. Actin reactivity was detected with a phosphatase alkaline-conjugated secondary antibody. Control experiments employing PPAR\(\gamma\) and PPAR\(\tau\) antibodies (Santa Cruz Biotechnology) were performed and no bands were detected in the presence of either antibody (data not shown). The relative intensity of protein signals was
quantified by densitometric analysis using the Sigma Gel Program (Sigma-Aldrich). Results are expressed as the ratio between the relative values of PPARδ and those of α-actin.

**Lipid level studies**

Seven embryos from each rat (n = 8 rats in each experimental group) were selected at random and incubated together in a metabolic shaker, under an atmosphere of 5% CO2 in 95% O2 at 37°C for 3 h in 1 ml KRB with or without the addition of cPGI2 (1 μM) (Cayman Chemical Co.), a stable PGI2 analogue that binds both membrane type PGI2 receptor and the nuclear receptor PPARδ (Forman et al., 1997). Embryos were also incubated in the presence of PGE1 (Cayman Chemical Co.), a cyclopentanone that activates PPARα (Yu et al., 1995). Embryo viability after the 3-h incubations was established by the presence of a beating heart. Concentrations of cPGI2 and PGE1 to be used were selected according to previous works evaluating the effect of different PGs and cyclopentenones in the embryo system and according to preliminary data that showed lower concentrations of the evaluated PGs and analogue geometries were devoid of the studied effects (Jawerbaum et al., 2002; Sinner et al., 2003).

After the embryo incubations in the presence of both cPGI2 and PGE1, embryos were stored at −70°C until determination of lipid levels by thin layer chromatography (TLC), as previously described (Jawerbaum et al., 2002). Briefly, the embryonic lipids were extracted in methanol–chloroform (2:1 v/v) and then concentrated in a Savant Speed-Vac concentrator. Total lipids were chromatographed with a solvent system consisting of hexane : ethyl ether : acetic acid at 80:20:2 v/v. After development, the TLC plate was dried for 5 min under a N2 stream and the lipids were stained with iodine vapours. Lipid species levels were quantified by comparison with known amounts of pure lipid standards run on the same plate. The plates were scanned and analysed by densitometry using the Sigma Gel Program (Sigma-Aldrich). Results are expressed as μg/mg protein.

**De novo lipid synthesis analysis**

Seven embryos from each rat (n = 8 rats in each experimental group) were selected at random and incubated together in a metabolic shaker, under an atmosphere of 5% CO2 in 95% O2 at 37°C for 3 h in 1 ml KRB with or without the addition of 14C-acetate (53 mCi/mmol) (Amersham Biosciences) added, and either with or without the addition of cPGI2 (1 μM) or PGE1 (2 μM). After incubations, embryos were stored at −70°C until determination of the newly formed radioactive lipids as previously described (Jawerbaum et al., 2002). Lipids were separated by TLC as described above. The radioactive spots corresponding to the different 14C-labelled lipid species were scraped into vials and counted in a liquid scintillation counter. Results are expressed as dpm/μg protein.

**Radioimmunoassay of PGE2**

Four embryos from each rat (n = 8 rats in each experimental group) were incubated together in a metabolic shaker, under an atmosphere of 5% CO2 in 95% O2 at 37°C for 3 h in 1 ml KRB with or without the addition of cPGI2 (1 μM) or PGE1 (2 μM). After incubations, both embryonic PGE2 content and PGE2 release to the incubating medium were analysed, as previously described (Jawerbaum et al., 2001). Briefly, to determine embryonic PGE2 content, embryos were homogenized and sonicated in PBS, an aliquot separated for protein determination by the Bradford method using a protein assay reagent (Bio-Rad Laboratories Inc.), and embryonic PGs were extracted twice in absolute ethanol. To determine embryonic PGE2 release to the incubating medium, we acidified the medium to pH 3.15 and extracted PGs three times with ethyl acetate. The extracts were dried under N2 atmosphere and stored at −70°C until radioimmunoassay, performed as previously described (Jawerbaum et al., 2001), employing specific antisera (Sigma-Aldrich). Sensitivities of these assays were 10 pg per tube. The cross-reactivity of PGE2 with other PGs was <0.1%. Results are expressed as pg/μg protein.

**Statistical analyses**

All data are presented as the mean ± SEM. Differences among groups were compared using Student’s t-tests, chi-square tests or ANOVA with Tukey’s post-hoc tests. A P value of <0.05 was considered statistically significant.

**Results**

**Embryo morphology, prostacyclin concentrations and protein expression of PPARδ**

In the experimental diabetic model evaluated, pregnant rats on day 10.5 of gestation showed marked hyperglycaemia (P < 0.001), increased resorption rate (P < 0.001) and increased malformation rate, mainly neural tube defects (P < 0.001), when compared with controls (Table I). In addition, embryos from diabetic rats at day 10.5 of gestation showed reduced somite number (P < 0.05) and diminished protein content, an index of growth delay (P < 0.01), when compared with embryos from control rats (Table I).

The concentrations of PGI2δ, an endogenous PPARδ agonist, measured by the determination of its stable metabolite 6-keto-PGF1α, were decreased in the embryos from diabetic animals when compared with controls (54%, P < 0.01) (Table I). In addition, the protein expression of PPARδ was also decreased in the embryos from diabetic animals when compared with controls (55%, P < 0.01) (Figure 1).

**cPGI2 effects on embryonic lipid levels and de novo lipid synthesis**

To analyse the influence of the activation of PPARδ on embryonic lipid levels, we cultured embryos obtained from control and diabetic rats on day 10.5 of gestation for 3 h in the presence or absence of the PPARδ agonist cPGI2 (1 μM), a stable analogue of prostacyclin that activates PPARδ, and then embryonic lipid levels were analysed. Consistent with our previous observations (Sinner et al., 2003), no differences were found when lipid levels were analysed both immediately and after the 3-h incubation. In addition, embryos from diabetic rats showed increased triglyceride levels and no changes in the concentrations of the other lipid species analysed, when compared with controls (Figure 2). Interestingly, we found that cPGI2 up-regulated the levels of phospholipids in embryos from control (50%, P < 0.001) and diabetic rats (54%, P < 0.01) (Figure 2). No significant changes in the levels of the other lipid classes were detected.

To further analyse whether these changes involved the modulation of de novo lipid synthesis, we cultured embryos obtained from control and diabetic rats for 3 h in the presence of 14C-acetate as a tracer, either with or without the PPARδ agonist cPGI2 (1 μM), and then the formation of radioactive lipids was analysed. As previously reported (Sinner et al., 2003), a clear reduction of de novo lipid synthesis of phospholipids, triglycerides, cholesterol and cholesteryl esters was detected in embryos from diabetic rats when compared with controls (Figure 3). Notably, we found that cPGI2 stimulated de novo lipid synthesis of phospholipids in embryos from both control (30%, P < 0.01)

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<tr>
<th>Table I. Maternal glycaemia and embryo morphology, growth and prostacyclin concentrations in control and diabetic rats on day 10.5 of gestation</th>
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<tr>
<td>Control (n = 10)</td>
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<tr>
<td>Rat glycaemia (mg/dl)</td>
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<td>Resorption rate</td>
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<td>Malformation rate</td>
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<td>Embryo somite number</td>
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<td>Embryo protein content (μg)</td>
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<td>Embryo PGE2 concentrations (pg/μg)</td>
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Chi-square statistics were performed on percentage values. Student’s t-tests were performed on means ± SEM data.

*P < 0.05, **P < 0.01 and ***P < 0.001 denote differences between control and diabetic groups.
and diabetic (70%, $P < 0.01$) rats (Figure 3). No changes in de novo synthesis of the other lipid classes evaluated were observed.

cPGI2 effects on embryonic PGE2 concentrations

To analyse the influence of the activation of PPARδ on embryonic PGE2 concentrations, we cultured embryos obtained from control and diabetic rats for 3 h in the presence or absence of the PPAR agonist cPGI1 (1 μM), and embryonic PGE2 content and release were evaluated. Consistent with our previous observations (Jawerbaum et al., 2001), embryos from diabetic rats showed decreased PGE2 content and increased PGE2 release to the incubation media, and PGE2 content values were similar when evaluated both before and after 3-h cultures. We found that in the embryos from control rats, cPGI1 significantly enhanced both PGE2 content (196%, $P < 0.01$) and release (297%, $P < 0.001$) (Figure 4). Moreover, in the embryos from diabetic rats, cPGI1 up-regulated PGE2 concentrations to control values (63% increase, $P < 0.05$) and further increased PGE2 release (79%, $P < 0.05$) (Figure 4).

Involvement of PPARδ on embryonic phospholipid levels and PGE2 concentrations

As a first step in elucidating the involvement of PPARδ activation in the observed cPGI2 effects, we used the cyclopentenone PGA1, another PPARδ activator that does not interact with the membrane type PGI1 receptors (Yu et al., 1995). Embryos obtained from control rats were cultured for 3 h in the presence or absence of the PPARδ agonist PGA1 (2 μM), and phospholipid and PGE2 concentrations were analysed. Similar to the results found with carboprostacyclin, we found that PGA1 additions up-regulated phospholipid levels (40%, $P < 0.05$) and de novo phospholipid synthesis (28%, $P < 0.05$). No changes in either the levels or de novo synthesis of the other lipid classes evaluated (triglycerides, cholesterol and cholesteryl esters) were observed in the presence of PGA1 (data not shown in figures). Interestingly, PGA1 markedly increased PGE2 content (393%, $P < 0.001$) and release of PGE2 (928%, $P < 0.001$) in rat embryos during early organogenesis (Figure 5).

Discussion

Embryonic malformations induced by maternal diabetes are mainly neural tube and cardiogenesis defects and arise during the highly susceptible period of early organogenesis (Mills et al., 1979; Eriksson et al., 2003; Jawerbaum and González, 2006). During organogenesis, lipids act as structural components and signalling molecules needed in appropriate locations at specific times to allow normal organ formation (Jollie, 1990; Herz and Farese, 1999). All three PPAR subtypes are important regulators of lipid homeostasis, but only PPARδ is present during rodent embryo early organogenesis (Braisant and Wahl, 1998). The findings of the present work demonstrate that PPARδ activation up-regulates phospholipid and PGE2 levels in rat embryos during early organogenesis and that these signalling pathways are likely to be impaired in the embryos from diabetic rats, because we observed reduced levels of PPARδ and its endogenous PPARδ activator PGA1.

Classically, PGI1 binds to membrane type PGI1 G-coupled membrane receptors. Renewed interest in this PG arose when it was found that it is also an endogenous agonist of the nuclear receptor PPARδ (Forman et al., 1997). cPGI1 rescues implantation defects in COX-2 (−/−) knockout mice through the activation of PPARδ (Lim and Dey, 2000). Also, adipogenesis differentiation mediated by PPARδ involves PPARδ activation (Xie et al., 2006). Impairment of PGI1 production in the diabetic pathology has been found in many different tissues in adults (White et al., 2002; Bolego et al., 2006), probably as a result of the inactivation of PGI1 synthase due to the formation of peroxynitrates (Zou et al., 1999), damaging agents formed by the interaction between NO and reactive oxygen species (ROS) and present in embryos from diabetic rats during early organogenesis (Jawerbaum et al., 2005). To our knowledge, this is the first report of impaired formation of PGI1 in embryos from diabetic mothers during organogenesis, an alteration that may impair functions mediated by both its membrane receptors and through PPARδ. Interestingly, in this work PPARδ protein expression was also found reduced in embryos from diabetic rats, although the complete understanding of the significance of this finding remains to be elucidated. PPARδ is the only PPAR isoform detected in rat embryos at the evaluated developmental stage both by western blot (unpublished results) and by in situ hybridization studies (Braisant and Wahl, 1998). PPARδ has been scarcely studied in diabetic pathology, but protein expression of PPARγ is decreased in placental tissues and skeletal muscle and increased in adipose tissues from diabetic patients (Jawerbaum et al., 2004; Carey et al., 2006).

PPARδ was first implicated in lipid metabolism in studies using knockout animals. Most PPARδ null embryos die during embry organogenesis due to a placental defect (Peters et al., 2000; Barak et al., 2002). The small percentage of PPARδ null mice that survive exhibit a reduction in fat mass, are smaller and have myelination defects in their brains (Peters et al., 2000).

Alterations in lipid levels and metabolism in the embryos from diabetic rats during early organogenesis have been previously reported (Jawerbaum et al., 2002; Sinner et al., 2003). An increased
accumulation of triglycerides, probably due to increased maternal transfer through the yolk sac, is found in the embryos from severe diabetic rats (Sinner et al., 2003). In embryos obtained from mild diabetic models, this increase is not observed, but a reduced de novo lipid synthesis is found in embryos from both mild and severe diabetic models during early organogenesis, probably to compensate the increase in lipids provided by the maternal environment (Jawerbaum et al., 2002). This reduction in the de novo lipid synthesis as a response of increased lipids from maternal origin is also observed later in gestation in both the fetuses and the placenta from diabetic rats (Capobianco et al., 2005). Decreased phospholipid synthesis has been previously related to fetal respiratory distress syndrome in maternal diabetes (Singh and Feigelson, 1983) and to the induction of malformations (Fisher et al., 2002). In this work, we found that cPGI2 up-regulated phospholipid levels in embryos from both control and diabetic rats. These effects seem to be dependent on PPARδ activation, because both cPGI2 and the cyclopentenone PGA1 stimulated with similar efficiencies not only phospholipid synthesis but also phospholipid synthesis during embryogenenesis. Previous studies have shown different effects of 15deoxyΔ12,14PGJ2, a cyclopentenone that reduces de novo lipid synthesis in embryos and placentas from both control and diabetic rats by both PPARγ-dependent and PPARγ-independent mechanisms (Jawerbaum et al., 2002; Capobianco et al., 2005). Although we found that phospholipid levels were unchanged in the embryos from diabetic rats, de novo synthesis of phospholipids was reduced, an alteration that may lead to changes in the localization of phospholipids in both surface and nuclear cell membranes and therefore in the availability of arachidonic acid, through the action of phospholipases. Lipid-signalling molecules are needed in the appropriate space and time location during embryo organogenesis. A reduction in the arachidonate-derived PGE2 within the embryos from diabetic rats has been clearly related to diabetic embryopathy (Wiznitzer et al., 1999; Jawerbaum and Gonzalez, 2005; Wentzel and Eriksson, 2005). Together with this reduction in intraembryonic PGE2 content, the embryo releases higher amounts of this PG, an alteration that probably contributes to the depletion of the needed arachidonate stores (Reece et al., 2006), and that may be the result of a transport defect, a membrane leak or a shift in the proportion of PGs retained and released by the embryo (Jawerbaum et al., 2000). COX-1 and COX-2 are expressed in both the nuclear envelope and the endoplasmic reticular
Figure 3. Effect of cPGI2 additions on de novo synthesis of (a) phospholipids, (b) triglycerides, (c) cholesterol and (d) cholesteryl esters in embryos obtained from control and diabetic rats on day 10.5 of gestation. Embryos were incubated with or without additions of cPGI2 1 μM for 3 h in Krebs Ringer Bicarbonate (KRB) in the presence of 1 μCi 14C-acetate (53 mCi/mmole) followed by evaluation of the incorporation of the tracer to lipids. Values are means ± SEM; n = 8 rats (56 embryos) per group. ANOVA followed by Tukey’s test was performed on the data. **P < 0.01 denotes differences between control groups with and without cPGI2 additions; ***P < 0.01 denotes differences between diabetic groups with and without cPGI2 additions; †P < 0.05 and †††P < 0.001 denote differences between control and diabetic groups.

Figure 4. Effect of cPGI2 additions on (a) PGE2 content and (b) PGE2 release in embryos obtained from control and diabetic rats on day 10.5 of gestation. Embryos were incubated with or without additions of cPGI2 1 μM for 3 h in Krebs Ringer Bicarbonate (KRB) followed by evaluation of PGE2 by radioimmunoassay. Values are means ± SEM; n = 8 rats (32 embryos) per group. ANOVA followed by Tukey’s test was performed on the data. **P < 0.01 and ***P < 0.001 denote differences between control groups with and without cPGI2 additions; #P < 0.05 denotes differences between diabetic groups with and without cPGI2 additions; †P < 0.05 and †††P < 0.001 denote differences between control and diabetic groups.
PGs synthesized in the latter exit cells and function through G-protein-coupled cell surface receptors. On the contrary, PGs synthesized in the nuclear membrane seem to function somewhat independently of the cell surface, with its own phospholipase A2 (to release arachidonic acid from nuclear envelope phospholipids), COXs and PG synthases (Marrache et al., 2005). As PGI2 synthase is localized in the nuclear membrane, PGI2 formation may be immediately available for its interaction with PPARδ (Lim and Dey, 2000). Interestingly, in this work we show that PPARδ agonists are important stimulators of PGE2 production that lead to increased embryonic PGE2 content and release. Although this effect has been observed in the presence of two PPARδ agonists, cPGI2 and PGA1, the higher efficiency of the latter suggests that a mechanism independent of PPARδ activation may also be involved in this stimulatory effect.

In diabetic embryopathy, a reduction in COX-2 levels has been related to the reduced embryonic PGE2 content (Wentzel et al., 1999) and may also be involved in the reduced PGI2 levels found in embryos from diabetic rats. Notably, we found in this work that the PPARδ agonist cPGI2 up-regulated the reduced PGE2 content in the embryos from diabetic rats and led to intraembryonic PGE2 concentrations similar to those found in control embryos. These effects may be the result of an up-regulation of the COX-2 gene, one of the identified PPAR downstream targets with a functional PPRE in its promoter (Meade et al., 1999). Further investigation is needed to identify the downstream genes regulated in the organogenetic embryo as a response to PPARδ, probably COX-2 and genes involved in phospholipid synthesis.

The results of this work provide novel evidence of PPARδ regulation of phospholipid and PGE2 levels during embryo early organogenesis and of an embryonic reduction in PPARδ and its agonist PGI2 due to maternal diabetes. These data raise the possibility of a model in which impaired activation of PPARδ may alter the lipid signalling required for normal organogenesis in embryos from diabetic mothers, and point to PPARδ as a putative target in diabetic embryopathy.

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