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Diminished PGE_2 content, enhanced PGE_2 release and defects in ^3H-PGE_2 transport in embryos from overtly diabetic rats

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Abstract. Diminished PGE_2 levels in diabetic embryos are related to the development of malformations, and thus the aim of the present study was to determine whether PGE_2 levels are modified in rat embryos cultured in diabetic serum during organogenesis, and if PGE_2 content and release, and ^3H-PGE_2 uptake and release, are altered in incubated diabetic embryos. Rats were made diabetic by steptozotocin (60 mg kg^{-1}) before mating. Control rat embryos cultured for 24 h (explantation Day 9) in the presence of diabetic serum showed diminished PGE_2 levels. When Day 10 diabetic embryos were incubated, embryo PGE_2 levels were lower, but the PGE_2 released to the incubation media was much higher than in controls. Uptake of ^3H-PGE_2 by diabetic embryos was initially enhanced (5–10 min), then reached similar levels to controls (20–100 min). Release of ^3H-PGE_2 previously incorporated during a 60-min incubation was greater in diabetic embryos than in controls. These results show diminished PGE_2 content in both diabetic and normal embryos cultured in the presence of diabetic serum, but suggest that diabetic embryos have the capability to produce and release high levels of PGE_2. The enhanced release of PGE_2 is probably the result of transport abnormalities, and leads to the elevated PGE_2 concentrations found in the incubating medium and to the diminished intraembryonic PGE_2 levels that alter embryonic development.

Extra keywords: diabetic embryopathy, malformations, pregnancy diabetes, prostaglandin E_2, rat embryo culture.

Introduction

Increased incidence of fetal malformation is a consequence of diabetic disease (Kitzmiller *et al*. 1978). Human congenital anomalies are induced before the 7th week of gestation, the teratogenic effects being greater in diabetic women with elevated levels of glycosylated haemoglobin at their initial prenatal evaluation and during the first trimester of pregnancy (Miller *et al*. 1988). Embryonic malformations similar to these described in humans have been found in animals in which the diabetic state has been induced chemically by streptozotocin (SZ-diabetic) (Uriu-Hare *et al*. 1985), but the mechanisms involved in the development of those anomalies are not completely understood. Experimental studies performed in embryos cultured *in vitro* during organogenesis have shown that diabetic serum, hyperglycaemia, low insulin levels and high concentrations of β-hydroxybutyrate induce dysmorphogenesis (Eriksson *et al*. 1991). Both an excess of radical oxygen species (ROS) and disturbed metabolism of arachidonic acid have been implicated (Wentzel *et al*. 1998). Antioxidants prevent embryonic malformations induced by hyperglycaemia *in vitro* (Eriksson and Borg 1993; Wentzel *et al*. 1997) and their administration to diabetic rats diminished embryonic abnormalities (Eriksson and Siman 1996; Viana *et al*. 1996). PGE_2 levels generated by the arachidonic acid cascade through cycloxygenase are diminished in SZ-diabetic embryos during early organogenesis, a decrease related to the presence of malformations (Piddington *et al*. 1996). Indeed, both arachidonic acid and PGE_2 supplementation protect against diabetic malformations *in vivo* (Goldman *et al*. 1985; Reece *et al*. 1996) and *in vitro* (Baker *et al*. 1990; Goto *et al*. 1992). Moreover, ROS and the arachidonic acid pathway are likely to be related, because PGE_2 levels are increased in the presence of the antioxidant N-acetylcysteine (Wentzel *et al*. 1999).

We recently described, in a type II diabetic rat model (neonatal-SZ model), the absence of embryonic malformations and enhanced PGE_2 production (Jawerbaum *et al*. 1998). As we had found previously in other tissues (Rettori *et al*. 1992; Herrero *et al*. 1995; Jawerbaum *et al*. 1997), embryonic PGE_2 levels are positively modulated by nitric oxide (Jawerbaum *et al*. 1998). Indeed, when both control and neonatal-SZ diabetic rats were treated with L-NAME, a nitric oxide inhibitor, embryonic PGE_2 generation diminished, and malformations developed in the embryos (Jawerbaum *et al*. 1998). Although it is known that prostaglandins are synthesized intracellularly and released near their sites of action before they are presented to membrane prostaglandin receptors (Smith 1989), they may have intracellular and nuclear functions (Lupulescu 1980). PGE_2 seems to be accumulated in the blastocyst prior to

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implantation (Jones and Harper 1984), and it has been found that uterine tissue accumulates and/or releases prostaglandins, according to different hormonal conditions (Gimeno *et al*. 1987; Motta *et al*. 1995). In view of these precedents, the aim of the present work was to evaluate (*1*) PGE_2 levels in normal embryos cultured *in vitro* in the presence of diabetic serum, (*2*) the relationship between the PGE_2 released into the incubating medium and the PGE_2 content in embryos obtained from control and SZ-diabetic rats and (*3*) the uptake and release of ^{3}H-PGE_2 by control and diabetic embryos and by normal embryos cultured in the presence of diabetic serum.

Materials and methods

Materials

Streptozotocin, citrate buffer, Tyrodes solution, culture-grade H_2O, penicillin, streptomycin, PGE_2 and rabbit antisera to quantify PGE_2 were all obtained from Sigma (St Louis, MO, USA). ^{3}H-PGE_2 and an Aquasol scintilliation cocktail were purchased from NEN Dupont (Boston, MA, USA).

Animals

Albino Wistar rats bred in our animal facility were fed Purina rat chow *ad libitum*. Female rats weighting 200–230 g were made diabetic by a single intraperitoneal injection of SZ (60 mg kg^{-1}) in citrate buffer (0.05 M, pH 4.5), as previously described (Jawerbaum *et al.* 1995). Control rats were injected with buffer alone. Five days after SZ administration, diabetic rats showing glycaemia greater than 400 mg dL^{-1} (Glucostix reagent strips, Bayer Diagnostics, Buenos Aires, Argentina) were considered diabetic. Oestrous cycles in the diabetic rats were observed during the two weeks after SZ administration, and during this period they became pregnant if mated. Normal and diabetic females were caged overnight with normal male rats and mating was confirmed by the presence of sperm in vaginal smears. When a positive pregnancy was identified, that was considered Day 0 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).

Whole embryo cultures

Animals were killed by cervical dislocation on Day 9 of pregnancy. The whole uterus was quickly removed and transferred to Petri dishes with Tyrodes solution. Using a stereomicroscope and watchmaker forceps, the balls of decidual tissues were removed from each uterus, and gently opened to free the conceptuses. Embryos were cultured *in vitro* for 24 h according to New (1978) in a medium comprising 75% rat serum (v/v) and 25% (v/v) Tyrodes solution. Culture media glucose levels were measured by an enzymatic colorimetric test (Wienner Lab, Argentina). Serum was collected from control and diabetic pregnant rats on Days 9–11 of gestation. Rats were anaesthetized with ether, underwent laparotomy and blood was collected from the abdominal aorta and centrifuged immediately. The resulting serum was pooled, and supplemented with penicillin (100 IU mL^{-1}) and streptomycin (100 µg mL^{-1}). The serum was stored frozen at –70°C until used. On the day of culture, the serum was thawed and heat-inactivated at 56°C for 45 min immediately before use. Three embryos were explanted and incubated in polypropylene tubes containing 3 mL of culture medium. Initially the culture flasks were gassed with a 5% O_2/5% CO_2 /90% N_2 mixture and incubated at 37–38°C on a rotary shaker operating at 30 r.p.m. After 16 h the flasks were gassed with 15% O_2/5% CO_2/80% N_2. At 24 h the embryos were harvested, the visceral yolk sac and amnion removed and the embryos evaluated morphologically. The embryonic heartbeat was used as a measure of viability. The embryos were categorized as morphologically normal or as showing neural tube defects or other malformations, such as microcephaly, abnormal cervical spine and tubular heart formation. Embryos were then sonicated in PBS to determine PGE_2 levels as described later.

Embryo incubation

Animals were killed by cervical dislocation on Day 10 of pregnancy, the uteri transferred to Petri dishes with Krebs–Ringer bicarbonate (KRB) solution (11.0 mM glucose, 145 mM Na^+, 2.2 mM Ca^{2+} , 1.2 mM Mg^{2+}, 127 mM Cl^-, 25 mM HCO_3^-, 1.2 mM SO_4^{2-} and 1.2 mM PO_4^{3-}) and the embryos separated from uterine tissue, decidua and embryonic membranes under a stereomicroscope. Embryos were evaluated morphologically as described above. Embryos in resorption stages were considered as resorbed and not analysed any further. Determination of embryonic PGE_2 content, PGE_2 release and ^{3}H-PGE_2 transport was performed as described below.

Prostaglandin determination

Embryonic PGE_2 content

PGE_2 content was quantified as previously performed (Motta *et al.* 1995) in embryos obtained after *in vitro* culture and from control and diabetic rats on Day 10 of gestation at time 0 or after 1 h incubation in KRB medium. Briefly, three embryos were sonicated in PBS, an aliquot separated for protein determination by Bradford's method (Bradford 1976), and embryonic PGE_2 was extracted twice in absolute ethanol. The extracts were dried under N_2 at room temperature, and stored at –70°C until radioimmunoassay (RIA) was performed.

Embryonic PGE_2 efflux

Three embryos were incubated together in a metabolic shaker, under an atmosphere of 5% CO_2 in 95% O_2 at 37°C, in 2 mL KRB medium for 1 h. After incubation, embryos were separated to measure PGE_2 content and proteins as described before, and the PGE_2 released to the incubation medium was quantified as previously described (Motta *et al.* 1995). Briefly, the incubation medium was acidified to pH 3.15 and extracted three times with ethyl acetate. The extracts were dried under N_2 at room temperature and stored at –70°C until RIA. PGE_2 was determined by RIA employing specific antisera from Sigma. Sensitivities of these assays were 10 pg $tube^{-1}$. The cross-reactivity of PGE_2 was 100% with PGE_1 and less than 0.1% with other prostaglandins. Results are expressed as pg (µg protein)$^{-1}$ (Figs 1 and 2) and pg $embryo^{-1}$ (Table 2).

^{3}H-PGE_2 transport

^{3}H-PGE_2 uptake and efflux studies were performed as described previously (Gimeno *et al.* 1987) in Day-10 embryos from control and diabetic rats, and in embryos cultured *in vitro* in the presence of control and diabetic serum that were obtained as described above.

Embryonic ^{3}H-PGE_2 uptake

Six embryos were incubated in the presence of 1.5 nM ^{3}H-PGE_2 (AE: 3.7 TBq mM NEN Dupont) in a metabolic shaker, in an atmosphere of 5% CO_2 in 95% O_2 at 37°C, in 2 mL KRB medium for different periods of time (5–100 min). At the end of the different incubation periods, the embryos were washed three times in 10 mL KRB to remove adherent ^{3}H-PGE_2 and dissolved in a vial containing the Aquasol scintilliation cocktail. Simultaneously, a 100-µl aliquot of the incubation medium was added to the vials. Radioactivity was determined in a scintilliation counter and results expressed as femtomols of ^{3}H-PGE_2 per mg protein per nanomolar concentration of ^{3}H-PGE_2. In order to validate the uptake experiments, similar experiments were

performed using ^{14}C-sucrose (AE: 7.4 GBq mM; NEN Dupont), an extracellular marker of molecular weight comparable to that of prostaglandins, and these showed no increases during 100 min of incubation (data not shown).

Embryonic ^{3}H-PGE_2 efflux

Six embryos were incubated in the presence of ^{3}H-PGE_2 (1.5 nM ^{3}H-PGE_2, AE: 3.7 TBq mM; NEN Dupont) for 60 min in a metabolic bath at 37°C. At the end of this period, the embryos were rapidly washed three times in 10 mL KRB, and transferred to separate tubes containing 2 mL KRB, which were incubated for 0, 5, 10, 15, 20 and 30 min respectively. At the end of the incubation periods, the embryos were transferred to vials containing the Aquasol scintillation fluid and the radioactivity was determined in a liquid scintillation counter. Results are expressed as percentage of remnant radioactivity at different times when related to Time 0 values.

Statistics

All values are presented as means ± SEM. Comparisons between groups were performed by one-way analysis of variance in conjunction with Tukey's test, Student's *t*-test or Chi-square test, where appropriate. Differences between groups were considered significant when $P = 0.05$ or less.

Results

Effects of overt diabetes and diabetic serum on embryo development and PGE_2 levels

Diabetic embryos obtained at Day 10 of gestation showed an increased resorption rate ($P<0.001$), an increased embryonic malformation rate ($P<0.001$), a diminished somite number ($P<0.05$) and diminished protein content (indicating growth delay, $P<0.01$) when compared with control embryos (Table 1). Normal embryos (explantation Day 9 of gestation) were cultured for 24 h in the presence of control (CS) or diabetic (DS) serum. Glucose concentration in culture medium containing DS was higher than in the medium containing CS (DS: 400 ± 44 mg dL^{-1} (n = 14); CS: 108 ± 13 mg dL^{-1} (n = 14); $P<0.001$). Control embryos cultured in the presence of DS showed an increased rate of malformed embryos ($P<0.05$), a diminished somite number ($P<0.01$) and diminished protein content ($P<0.01$) when compared with those found in embryos cultured in the presence of CS (Table 1).

PGE_2 content was measured in control, diabetic and *in vitro*-cultured embryos, and was found to be diminished in the embryos obtained from diabetic rats (Day 10 of gestation, $P<0.01$) as compared with controls (Fig. 1), and in the normal embryos cultured in the presence of DS ($P<0.05$) when compared with the embryos cultured in the presence of CS (Fig. 1).

Relationship between PGE_2 accumulation and release in control and diabetic embryos

Embryos from control and SZ-diabetic rats on Day 10 of gestation were incubated for 1 h in KRB medium in a shaking bath under an atmosphere of 95% O_2/5% CO_2 at 37°C. After incubation, the concentration of PGE_2 released into the incubation medium and the embryonic PGE_2 content were determined. PGE_2 was mostly released into the incubation medium, since the values for PGE_2 in the media were much higher than those for PGE_2 remaining in the embryos (Fig. 2). The diabetic embryos released more PGE_2 than the

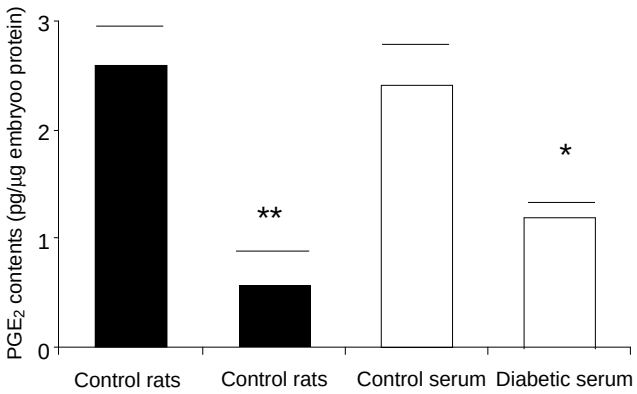

Fig. 1. PGE_2 content in embryos from control and diabetic rats on Day 10 of gestation (■) and from embryos explanted on Day 9 of gestation and cultured *in vitro* during 24 h in the presence of serum from control and diabetic rats (□). Values are mean and SEM for 30–33 embryos obtained randomly from 9–10 rats. $^{**}P<0.01$, $^{*}P<0.05$ (ANOVA, Tukey's test).

Table 1. Outcome of embryos from control and diabetic rats (Day 10 of gestation), and from control embryos (explantation Day 9 of gestation) after 24 h culture in the presence of serum from control and diabetic rats

	Embryos			
	From control rats (n)	From diabetic rats (n)	Cultured in control serum (n)	Cultured in diabetic serum (n)
Resorptions	0% (0/93)	20% (17/85)[a]	–	–
Malformations	1% (1/93)	20% (14/68)[a]	4% (3/75)	16% (13/79)[b]
Somite number	11 ± 0.3 (93)	9 ± 0.5 (68)[c]	12 ± 0.7 (75)	9 ± 0.7 (79)[d]
Protein content (µg)	66 ± 4 (19)	35 ± 3 (18)[e]	63 ± 4 (19)	41 ± 5 (19)[d]

Values are percentage of embryos (Chi-square) or means ± SEM (ANOVA, Tukey's test) for embryos obtained randomly from 10–12 rats. [a]$P<0.001$ *v*. embryos from control rats, [b]$P<0.05$ *v*. embryos cultured in control serum, [c]$P<0.05$ *v*. embryos from control rats, [d]$P<0.01$ *v*. embryos cultured in control serum, [e]$P<0.01$ *v*. embryos from control rats.

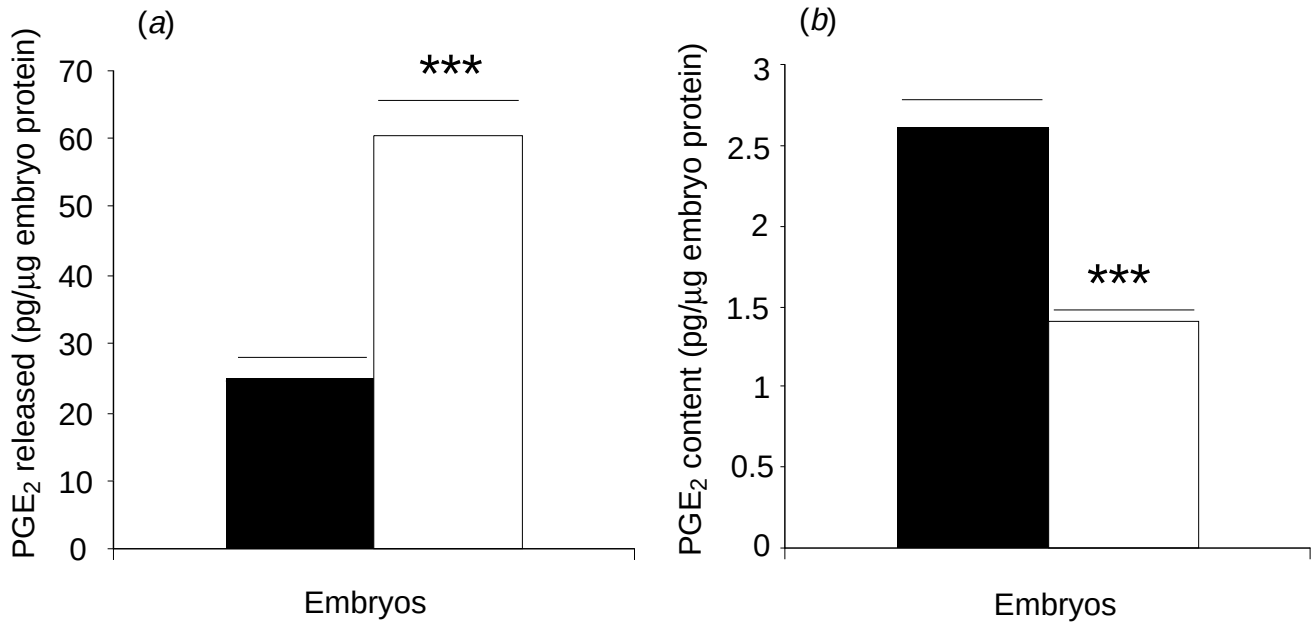

Fig. 2. (*a*). PGE_2 released to the incubating medium by embryos from control (■) and diabetic (□) rats on Day 10 of gestation, after 1 h incubation in KRB medium. Values are means and SEM for 30–33 embryos obtained randomly from 9–10 rats. ***P<0.001 (Student's t-test). (*b*) PGE_2 content in embryos from control (■) and diabetic (□) rats on Day 10 of gestation, after 1 h incubation in KRB medium. Values are means and SEM for 30–33 embryos obtained randomly from 9–10 rats. ***P<0.001 (Student's t-test).

normal embryos (P<0.001, Fig. 2*a*). A diminished PGE_2 content was found in diabetic embryos after incubation (P<0.001, Fig. 2*b*) as compared with control embryos. The total amount of PGE_2 produced by the diabetic embryo during the incubation time was enhanced when compared with that generated by the control embryo (P<0.02, Table 2). Indeed, control embryo production of PGE_2 during incubation was 10 times its initial value, whereas the diabetic embryo generated, in the same period, 100 times the initial PGE_2 content (Table 2).

Table 2. PGE_2 production by control and diabetic embryos (Day 10 of gestation), after 1 h incubation in KRB medium

	Control	Diabetic
Embryonic PGE_2 content before incubation (pg embryo^{-1})	175 ± 13	19.9 ± 0.5[a]
Total amount of PGE_2 after incubation (embryo content + PGE_2 in incubation medium; pg embryo^{-1})	1573 ± 140	2282 ± 230[b]
PGE_2 generated per embryo	10× initial value	100× initial value

Values are means ± SEM (ANOVA, Tukey's test) for embryos obtained randomly from 10 rats. [a]P<0.001 *v*. embryos from control rats, [b]P<0.02 *v*. embryos from control rats.

Uptake and release of ^{3}H-PGE_2 in control and diabetic embryos

Accumulation of ^{3}H-PGE_2 by control and diabetic embryos (Day 10 of gestation) was evaluated during 100 min of incubation. There was enhanced uptake of ^{3}H-PGE_2 by diabetic embryos at 5 and 10 min (P<0.01) of incubation as compared with controls. Afterwards, no difference in ^{3}H-PGE_2 uptake was found between control and diabetic embryos (Fig. 3).

Control and diabetic embryos were pre-incubated for 60 min with ^{3}H-PGE_2 and then release of ^{3}H-PGE_2 was determined for a period of 30 min. There was enhanced release by diabetic embryos when compared with controls from 10 to 30 min of incubation (P<0.05, Fig. 4). Uptake and release of ^{3}H-PGE_2 were evaluated in control embryos cultured *in vitro* in the presence of CS and DS: no differences in uptake were found after 60 min of incubation, but enhanced release of the previously incorporated ^{3}H-PGE_2 was found after 30 min of incubation by embryos cultured in the presence of DS when compared with those cultured in the presence of CS (P<0.05, Table 3).

Discussion

Accumulating evidence suggests that the arachidonic acid–prostaglandin pathway is involved in diabetic embryopathy. Several published studies demonstrate that arachidonic acid supplementation can reverse many mor-

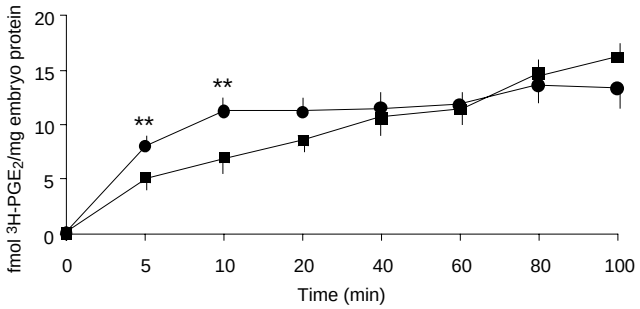

Fig. 3. Uptake of ^{3}H-PGE_2 during 100 min incubation by embryos from (■) control and (●) diabetic rats (Day 10 of gestation). Values are means ± SEM for 20–22 embryos obtained randomly from 10–11 rats. **P<0.01 *v*. embryos from control rats (ANOVA, Tukey's test).

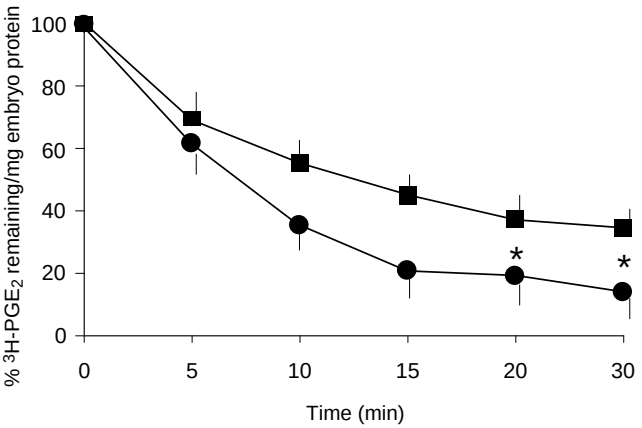

Fig. 4. Release of ^{3}H-PGE_2 by embryos from (■) control and (●) diabetic rats (Day 10 of gestation) at different times, after being preincubated for 60 min in the presence of ^{3}H-PGE_2. Results are expressed as % of ^{3}H-PGE_2 remaining in the embryo. Values are means ± SEM for 20–22 embryos obtained randomly from 10–11 rats. *P<0.05 *v*. embryos from control rats (ANOVA, Tukey's test).

Table 3. Uptake and release of ^{3}H-PGE_2 by embryos explanted on Day 9 and cultured *in vitro* during 24 h in the presence of serum from control and diabetic rats

	Control serum	Diabetic serum
Uptake of ^{3}H-PGE_2 (fmol ^{3}H-PGE_2/mg embryo protein)	11 ± 1	12 ± 2
Release of ^{3}H-PGE_2 (%^{3}H-PGE_2 remaining in embryo/mg embryo protein)	49 ± 5	29 ± 5[a]

Uptake of ^{3}H-PGE_2 was evaluated in embryos after 60 min incubation. Release of ^{3}H-PGE_2 was evaluated in embryos at 30 min, after a 60-min loading with ^{3}H-PGE_2 (results are expressed as % of ^{3}H-PGE_2 remaining in the embryo). Values are means ± SEM (Student's *t*-test) for 20–22 embryos obtained randomly from 9–10 rats. [a]P<0.02 *v.* embryos cultured in control serum.

phological and biochemical changes associated with diabetes-induced malformations in both the embryo and yolk sac (Goldman *et al.* 1985; Pinter *et al.* 1986; Reece *et al.* 1996). PGE_2, a major metabolite of arachidonic acid, can also reverse glucose-induced teratogenicity *in vitro* (Baker *et al.* 1990; Goto *et al.* 1992). As previously described in embryos from diabetic mice (Piddington *et al.* 1996) and in control embryos cultured *in vitro* during organogenesis in hyperglycaemic conditions (Wentzel *et al.* 1999), we have found in this work that serum from overtly diabetic rats induces malformations and diminishes embryonic PGE_2 levels. Levels of PGE_2 in the mice embryos were higher during early neurulation and diminished when it was complete, suggesting that this pathway is important during neural tube closure (Piddington *et al.* 1996). We have also evaluated the embryonic capacity for generating PGE_2 during neurulation in experiments performed during a 1-h incubation (Jawerbaum *et al.* 1998), in which PGE_2 metabolism proceeded at a very low rate (Klein *et al.* 1981; Durant *et al.* 1988). PGE_2 was produced and released in high amounts by the control embryos during organogenesis. Diabetic embryos had diminished intraembryonic levels of PGE_2 when compared with controls, but we found that they released more PGE_2 into the incubating medium. This indicates that diabetic embryos can produce PGE_2, and in even greater amounts than controls.

In order to understand why intraembryonic levels of PGE_2 are diminished in the diabetic embryo in spite of the enhanced PGE_2 generation, we evaluated ^{3}H-PGE_2 uptake and release in control and diabetic embryos and in embryos cultured in the presence of DS. Initially, increased ^{3}H-PGE_2 uptake was found in diabetic embryos, probably to compensate for the initial low PGE_2 levels. No alteration in ^{3}H-PGE_2 uptake was detected in diabetic embryos after 10 min. After 60 min of incubation in the presence of ^{3}H-PGE_2, diabetic embryos showed enhanced release to the incubating medium. The increased release of ^{3}H-PGE_2 and the diminished intraembryonic PGE_2 levels found in diabetic embryos, even when the synthesis of this prostaglandin is increased, suggests that either a membrane leak or increased transport of this prostaglandin occurs in diabetic embryonic cells. In the present work, we found diminished embryonic PGE_2 content and also increased release of ^{3}H-PGE_2 in embryos cultured in the presence of DS. As hyperglycaemia has been previously shown to induce diminished PGE_2 levels and dysmorphogenesis (Wentzel *et al.* 1999), it is likely that it induces the changes found in the embryos cultured in the presence of DS. PGE_2 uptake and release seems to be dependent on the prostaglandin transporter (PGT), which has a high affinity for PGE_1, PGE_2 and $PGF_{2\alpha}$ and is a cotransporter that modulates release and/or uptake of these prostaglandins, apparently favouring one of these mechanisms depending on the concentration of an unidentified

metabolite that is cotransported (Chan *et al.* 1998). PGT is present in most tissues (Kanai *et al.* 1995), but its expression is unknown in the developing embryo. Future research is needed to evaluate the involvement of PGT in the altered PGE_2 transport in the diabetic embryo. In embryos from the neonatal-SZ rat model of type II diabetes, we have previously found not only an enhanced release of PGE_2, but also an elevated intraembryonic level of PGE_2, which is probably related to the absence of embryonic malformations in this model (Jawerbaum *et al.* 1998).

In overtly diabetic rats, serum arachidonate levels were found to be diminished during pregnancy (Reece *et al.* 1996), and these values were related to abnormal embryonic development and altered function of the yolk sac, the latter having a critical role in selective transfer of macromolecules to the developing embryo (Pinter *et al.* 1986). Indeed, the uptake of arachidonic acid by embryonic yolk sacs has been found to be increased in embryos cultured in hyperglycaemic conditions (Engstrom *et al.* 1991). It is interesting to speculate that arachidonic acid might be depleted if PGE_2 generation and release is increased in the diabetic embryo in order to maintain intracellular PGE_2 levels. Prostaglandin generation induces free oxygen radicals formation (Kukreja *et al.* 1986), a mechanism that seems to mediate ROS generation in different diabetic tissues (Williamson *et al.* 1993). Indeed, oxidative damage to proteins and to DNA, generated by enhanced prostaglandin H synthase activation, has been proposed as a mechanism mediating phenytoin and benzo[a]pyrene teratogenesis (Winn *et al.* 1997). Moreover, there is a putative common mechanism between diabetic dysmorphogenesis induced by PGE_2 and by ROS, because N-acetylcysteine addition prevents the decrease of embryonic PGE_2 content and prevents malformations induced by hyperglycaemia (Wentzel *et al.* 1999).

In the present work, we found that PGE_2 levels are diminished in diabetic embryos and embryos cultured *in vitro* in the presence of serum from overtly diabetic rats. Interestingly, our results show that diabetic serum *in vitro* and the diabetic environment *in vivo* cause enhanced release of PGE_2 by the embryo, suggesting that the embryo is capable of producing increased amounts of PGE_2, but is not able to maintain intraembryonic PGE_2 levels. Thus it is likely that PGE_2 is needed inside the cell to modulate embryonic growth and development. Moreover, diminished levels of embryonic PGE_2 may stimulate the production of this prostaglandin, probably leading to ROS generation and inducing protein and DNA damage. In addition, the enhanced PGE_2 synthesis may produce arachidonate depletion in the diabetic embryo, leading to alterations in the cell membrane and in the structure and function of yolk sac. In conclusion, this study suggests that both deficiency of endogenous PGE_2 and enhanced PGE_2 production in the diabetic embryo are related to dysmorphogenesis.

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References

Baker, K., Piddinton, R., Goldman, A., Egler, J., and Moerhring, J. (1990). Myoinositol and prostaglandins reverse the glucose inhibition of neural tube fusion in cultured mouse embryos. *Diabetologia* **33**, 593–6.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–54.

Chan, B., Satriano, J., Pucci, M., and Shuster, V. (1998). Mechanism of prostaglandin E_2 transport across the plasma membrane of HeLa cells and xenopus oocytes expressing the prostaglandin transporter 'PGT'. *J. Biol. Chem.* **273**, 6689–97.

Durant, S., Dubal, D., Homo-Delarche F. (1988). Mouse embryo fibroblasts in culture: characteristis of arachidonic acid metabolism during early passages. *Prostaglandins Leukotrienes Essent. Fatty Acids* **32**, 129–37.

Engstrom, E., Haglund, A., and Eriksson, U. J. (1991). Effects of maternal diabetes or *in vitro* hyperglycemia on uptake or palmitic and arachidonic acid by rat embryos. *Pediatr. Res.* **30**, 150–3.

Eriksson, U. J., and Borg, K. (1993). Protection by free oxygen radical scavenging enzymes against glucose-induced embryonic malformations *in vitro*. *Diabetologia* **34**, 325–31.

Eriksson, U. J., and Siman, C. M. (1996). Pregnant rats fed the antioxidant butylated hydroxytoluene show decreased occurrence of malformations in offspring. *Diabetes* **45**, 1497–502.

Eriksson, U. J, Borg, H., Fordberg, H., and Styrud, J. (1991). Diabetic embryopathy: Studies with animal and *in vitro* models. *Diabetes* **40**, 94–7.

Gimeno, M. A. F., Franchi, A. M., Gonzalez, E. T., and Gimeno, A. L. (1987). Uptake and release of ^3H prostaglandins E_2 and $F_{2\alpha}$ in uterine strips isolated from ovariectomized rats: Influence of *in vitro* progesterone. *Prostaglandins* **33**, 51–61.

Goldman, A., Baker, L., Piddington, R., Marx, B., Herold, R., and Egler, J. (1985). Hyperglycemia-induced teratogenesis is mediated by a functional deficiency of arachidonic acid. *Proc. Natl Acad. Sci. USA* **82**, 8227–31.

Goto, M. P., Goldman, A. S., and Uhing, M. R. (1992). PGE_2 prevents anomalies induced by hyperglycemia or diabetic serum in mouse embryos. *Diabetes* **41**, 1644–50.

Herrero, M. B., Franchi, A. M., and Gimeno, M. A. F. (1995). Mouse spermatozoa can synthesize PGE and 5HETE *in vitro*: stimulatory action of nitric oxide. *Prostaglandins Leukotrienes Essent. Fatty Acids.* **56**, 347–50.

Jawerbaum, A., Franchi, A. M., Gonzalez, E. T., Novaro, V., and Gimeno, M. A. F. (1995). Hyperglycemia promotes elevated generation of TXA_2 in isolated rat uteri. *Prostaglandins* **50**, 47–56.

Jawerbaum, A., Gonzalez, E. T., Faletti, A., Novaro,V., and Gimeno, M. A. F. (1997). Nitric oxide mediates hCG induced prostaglandin E generation in rat oocyte–cumulus complexes. *Reprod. Fertil. Dev.* **9**, 391–4.

Jawerbaum, A., Gonzalez, E. T., Novaro, V., Faletti, A., Sinner, D., and Gimeno, M. A. F. (1998). Increased prostaglandin E generation and enhanced nitric oxide synthase activity in the non-insulin-dependent diabetic embryo during organogenesis. *Reprod. Fertil. Dev.* **10**, 191–6.

Jones, M., and Harper, M. (1984). Rabbit blastocysts accumulate ^{3}H prostaglandins *in vitro. Endocrinology* **115**, 817–23.

Kanai, N., Lu, R., Satriano, J., Bao, Y., Wolkoff, A., and Shuster, V. (1995). Identification and characterization of a prostaglandin transporter. *Science* **268**, 866–9.

Kitzmiller, J. L., Cloherty, J. P., Younger, M. D., Rothchild, S. B., Sosenko, I., Epstein, M. F., Singh, S., and Neff, R. K. (1978). Diabetic pregnancy and perinatal morbidity. *Am. J. Obstet. Gynecol.* **131**, 560–80.

Klein, K. L., Scott, W. J., Clark, K. E. (1981). Measurements of prostaglandins in embryonic tissue using radioimmunoassay. *Prostaglandins* **22**, 623–30.

Kukreja, R. C., Kontos, H. A., Hess, M. L., and Ellis, E. F. (1986). PGH synthase and lipoxygenase generate superoxide in the presence of NADH or NADPH. *Circ. Res.* **59**, 612–19.

Lupulescu, A. (1980). Heavy incorporation of ^{3}H prostaglandin F_{2alpha} in the neoplastic cells as revealed by autoradiographic studies. *Experientia* **36**, 246–7

Miller, E., Hare, J. W., Cloherty, J. P., Garcia-Segura, M., Hobbins, J. C., Mahoney, M. J., and Naftolin, F. (1988). Elevated maternal hemoglobin a in early pregnancy and major congenital anomalies in infants of diabetic mothers. *N. Engl. J. Med.* **304**, 1331–4

Motta, A., Faletti, A., and Gimeno, M. A. F. (1995). Influence of progesterone levels on prostaglandin concentrations in isolated uterine tissue and incubation medium from pseudopregnant rats. *Prostaglandins* **50**, 213–23

New, D. A. T. (1978). Whole embryo culture and the study of mammalian embryos during organogenesis. *Biol. Rev.* **53**, 81–122

Piddington, R., Joyce, J., Dhanasekaran, P., and Baker, L. (1996). Diabetes mellitus affects prostaglandin E2 levels in mouse embryos during neurulation. *Diabetologia* **39**, 915–20.

Pinter, E., Reece, A., Leranth, C. *et al.* (1986). Arachidonic acid prevents hyperglycemia-associated yolk sac damage and embryopathy. *Am. J. Obstet. Gynecol.* **155**, 691–702.

Reece, A., Wo, Y., Wiznitzer, A., Homko, C., Yao, J., Borenstein, M., and Sloskey, G. (1996). Dietary polyunsaturated fatty acids prevents malformations in offspring of diabetic rats. *Am. J. Obstet. Gynecol.* **175**, 818–23.

Rettori, V., Gimeno, M. A. F., Lyson, K., and McCann, S. M. (1992). Nitric oxide mediates norepinephrine induced prostaglandin E_2 release from the hypothalamus. *Proc. Natl Acad. Sci. USA* **89**, 11543–4.

Smith, W. L. (1989). The eicosanoids and their biochemical mechanisms of action. *Biochem. J.* **259**, 315–24.

Uriu-Hare, J. Y., Stern, J. S., Reaven, G. M., and Keen, C. L. (1985). The effect of maternal diabetes on trace element status and fetal development in the rat. *Diabetes* **34**, 1031–40.

Viana, M., Herrera, E., and Bonet, B. (1996). Teratogenic effects of diabetes mellitus in the rat: prevention with vitamin E. *Diabetologia* **39**, 1047–54.

Wentzel, P., and Eriksson, U. J. (1998). Antioxidants diminish developmental damage induced by high glucose and cyclooxygenase inhibitors in rat embryos *in vitro*. *Diabetes* **47**, 677–84.

Wentzel, P., Thunberg, L., and Eriksson, U. J. (1997). Teratogenic effect of diabetic serum is prevented by supplementation of superoxide dismutase and N-acetylcysteine in rat embryo culture. *Diabetologia* **40**, 7–14.

Wentzel, P., Welsh, N., and Eriksson, U. J. (1999). Developmental damage, increased lipid peroxidation, diminished cyclooxygenase-gene expression and lowered prostaglandin E2 levels in rat embryos exposed to a diabetic environment. *Diabetes* **48**, 813–20.

Williamson, J., Chang, K., Frangos, M., Hasan, K. D., Ido, Y., Kawamura, T., Nyengaard, J. R., Vand der Enden, M., Kilo, C., and Tilton, R. G. (1993). Hyperglycemic pseudohypoxia and diabetic complications. *Diabetes* **42**, 801–13.

Winn, L. M., and Wells, P. G. (1997). Evidence for embyonic prostaglandin H synthase-catalyzed bioactivation and reactive oxygen species-mediated oxidation of cellular macromolecules in phenytoin and benzo[a]pyrene teratogenesis. *Free Radicals Biol. Med.* **22**, 607–21.

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