Effects of prostaglandins E-2 and F-2 α on the metabolism of [U-¹⁴C]glucose by mouse morulae–early blastocysts *in vitro*

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Summary. During 5-h culture in the presence of radioactive glucose, PGE-2 (10 μ g/ml) significantly inhibited incorporation of glucose into the acid-soluble glycogen pool. PGE-2 at 1 and 10 μ g/ml and PGF-2 α at 1 μ g but not 10 μ g/ml stimulated incorporation of glucose into non-glycogen macromolecules during culture. However, the utilization of acid-soluble glycogen and other biochemical pools was not affected by the presence of PGs in the medium during 24-h chase culture of pulse-labelled embryos. Carbon dioxide production was significantly suppressed in the presence of PGs but accumulation of lactate was not affected. The results indicate that PGE-2 and PGF-2 α , in physiological concentrations, directly influence the metabolism of glucose by preimplantation embryos.

Introduction

There are several reports of the effects of prostaglandins (PGs) on embryos. Based on an electron microscopic study, Holmes & Gordashko (1980) reported that both PGE-2 and PGF-2 α stimulate changes in the trophoblast cells of the blastocyst and activate the blastocyst to implant in the uterus. Holmes & Gordashko (1980) also observed that blastocysts prevented from implanting by indomethacin were impeded in their morphological activation. More directly, several PG antagonists have been shown to inhibit hatching of mouse blastocysts *in vitro* and it has been suggested that PGs are necessary for the accumulation of fluid in the blastocyst (Biggers *et al.*, 1978). RNA synthesis by mouse embryos is also affected directly by PGF-2 α (Uehara *et al.*, 1984).

It has been demonstrated that PGs can affect the carbohydrate metabolism of cells (Robertson, 1979) and that indomethacin can modulate glycogen content (Spatz *et al.*, 1986). Ozias & Stern (1973) reported that there were differences in the glycogen content of cultured and freshly collected mouse embryos at the late preimplantation stage. In an attempt to explain this discrepancy, Edirisinghe & Wales (1984) reported increased degradation of acid-soluble glycogen of pulse-labelled embryos on transfer to ovariectomized mice receiving progesterone, but more recent studies indicate that this increased degradation of glycogen was not due to the direct action of ovarian steroids on embryos (Khurana & Wales, 1987).

It has been suggested that PGs may act as an intermediary in the action of sex steroids on the blastocyst–endometrial system (Ham *et al.*, 1975; Holmes & Gordashko, 1980) and a high local concentration of PGs has been found at implantation sites (Kennedy, 1977). The evidence that uterine PGs are transported directly to the ovary to cause luteolysis (McCracken, 1971) suggests that they cross membranes easily and so PGs synthesized by uterine tissue most probably pass into the uterine lumen (Demers *et al.*, 1974).

The present experiments were performed to determine the direct effects of PGE-2 and PGF-2 α on glucose metabolism by mouse morulae–early blastocysts.

Materials and Methods

General

Randomly bred, 7–8-week-old Swiss albino mice of the Quackenbush (Q(s)) strain were used as a source of embryos. The mice were induced to superovulate by injections of PMSG and hCG and embryos at the morula–early blastocyst stage were collected 96 h after hCG injection (Pike & Wales, 1982). Medium based on Krebs–Ringer–bicarbonate solution containing 25 mM-lactate, 0.25 mM-pyruvate, 1 mg bovine serum albumin/ml (Commonwealth Serum Laboratories, Melbourne, Australia) and antibiotics (Brinster, 1965) was used for the collection of embryos. Studies of the incorporation and turnover of glucose were undertaken using the above medium supplemented with [U-¹⁴C]glucose. For the studies of the catabolic utilization of glucose, lactate and pyruvate were omitted from the medium and [U-¹⁴C]glucose was added as the sole energy substrate. For each replicate of an experiment, embryos were flushed from the uterine horns of the mice, placed in a common pool and washed by transfer through fresh medium before being distributed randomly amongst the different treatment groups within 30 min of collection. The mean number of embryos used per treatment group ranged from 25 to 50. All incubations were carried out at 37°C in a humidified atmosphere of 5% CO₂ in air.

Media containing prostaglandins

PGE-2 and PGF-2 α (Sigma Chemical Company, St Louis, MO, U.S.A.) were dissolved in culture medium just before use and diluted to obtain concentrations of 1 μ g/ml and 10 μ g/ml. Because of the lipid solubility of these compounds, all incubations of embryos to test the effects of PGs (including controls) were carried out in glass tubes (12 $\times$ 100 mm) containing 0.7 ml medium rather than in microdroplets of medium under paraffin oil.

Isotopic labelling of embryos

Incorporation studies. The effect of PGE-2 and PGF-2 α on incorporation of glucose carbon into different biochemical fractions of preimplantation embryos was studied by incubating embryos for 5 h in 0.7 ml medium containing 0.28 mM-[U-¹⁴C]glucose (sp. act. 1.11 MBq/ μ mol; Radiochemical Centre, Amersham, U.K.) with or without the addition of PGs. After culture, embryos were washed twice and stored at –70°C for biochemical fractionation.

Turnover studies. Embryos were pulse-labelled for 2 h by culturing in microdroplets of medium under oil, the medium containing 0.28 mM-[U-¹⁴C]glucose (sp. act. 1.11 MBq/ μ mol). After pulse culture, a random sample (~16%) of the washed embryos was collected in a small volume (<5 μ l) and stored at –70°C for subsequent fractionation to estimate incorporation during the 2-h pulse. The remaining embryos were chase-cultured in tubes for 24 h in 0.7 ml medium with or without PG. After chase culture, embryos were washed twice through 2 ml PG free medium containing unlabelled glucose and stored at –70°C until fractionation.

Measurement of catabolic utilization of glucose

The production of CO₂ and lactate from glucose was used to assess the catabolic utilization of substrate (Khurana & Wales, 1987). In summary, 10 embryos were placed in 50 μ l medium with 0.28 mM-[U-¹⁴C]glucose (sp. act. 1.11 MBq/ μ mol) as sole energy substrate and incubated for 4 h in a sealed vial. Culture was terminated by the injection of acid and CO₂ produced was trapped with alkali. Lactate accumulated in the medium was separated from the parent substrate by paper chromatography using butanol:acetic acid:water (4:1:5, by vol.) as solvent. The radioactivity of the CO₂ produced and lactate accumulated was converted to picomoles of metabolite per embryo per hour after taking into account specific activity, machine efficiency and background counts.

Fractionation of embryos

Labelled embryos were thawed and processed within 1 week of the completion of an experiment, using the method described previously (Edirisinghe *et al.*, 1984). Four biochemical fractions (acid-soluble glycogen, non-glycogen acid-soluble fraction, acid-insoluble glycogen and non-glycogen acid-insoluble fraction) were isolated. The radioactivity in each fraction was determined by the use of scintillation spectrometry and the value for pg-atoms of glucose carbon incorporated was calculated from the disintegrations per minute and the specific activity of parent substrate.

Statistical analysis

After logarithmic transformation to equalize variances, the statistical significance of effects was assessed by standard analysis of variance using orthogonal polynomials to test factorial comparisons. To assess the statistical significance of treatment effects compared to controls, comparisons were made using the Q test for all comparisons between means (Snedecor & Cochran, 1967).

Results

The data from the experiment to test the effects of PGE-2 and PGF-2 α on the incorporation of glucose carbon by morulae–early blastocysts during 5-h culture are presented in Table 1. Statistical analysis of the results revealed that the addition of prostaglandins had no effect on the incorporation of glucose carbon into either the non-glycogen acid-soluble or the acid-insoluble glycogen fraction, but there were significant treatment effects for the other two fractions ($P < 0.01$). Compared to control, incorporation into acid-soluble glycogen was significantly suppressed by the addition of 10 μ g PGE-2/ml to the incubation medium but was unaffected by the lower concentration of this compound, or by PGF-2 α . Inclusion of PGE-2 in the medium significantly stimulated incorporation into the non-glycogen acid-insoluble fraction in a dose-dependent manner. Furthermore, 1 μ g PGF-2 α /ml also stimulated incorporation into this fraction but the effect was lost when the concentration of this compound was raised to 10 μ g/ml.

The effect of PGE-2 and PGF-2 α on the turnover of glucose carbon is shown in Table 2. The amount of glucose carbon in the acid-soluble glycogen fraction was not affected by chase culture, but label in all other biochemical fractions isolated decreased significantly during chase. Among

Table 1. The effects of PGE-2 and PGF-2 α on the incorporation of glucose carbon into biochemical fractions of mouse morulae–early blastocysts during 5-h culture in a medium containing 0.28 mM-[U- 14 C]glucose, 25 mM-lactate and 0.25 mM-pyruvate

Treatment	Incorporation of glucose carbon (pg-atoms/embryo) into:			
	Acid-soluble fraction		Acid-insoluble fraction	
	Glycogen	Non-glycogen	Glycogen	Non-glycogen
None (control)	27.87 $\pm$ 2.48	12.18 $\pm$ 1.08	7.46 $\pm$ 1.21	7.50 $\pm$ 0.25
PGE-2 (1 μ g/ml)	25.88 $\pm$ 0.94	12.67 $\pm$ 0.82	6.58 $\pm$ 0.28	8.16 $\pm$ 0.28
PGE-2 (10 μ g/ml)	17.09 $\pm$ 1.94	13.65 $\pm$ 0.72	5.45 $\pm$ 0.57	8.76 $\pm$ 0.31
PGF-2 α (1 μ g/ml)	26.24 $\pm$ 1.80	13.50 $\pm$ 2.36	6.86 $\pm$ 1.06	8.15 $\pm$ 0.18
PGF-2 α (10 μ g/ml)	29.44 $\pm$ 1.02	11.79 $\pm$ 2.54	6.63 $\pm$ 0.35	7.68 $\pm$ 0.17

Values are means $\pm$ s.e.m. for three replicates.

Table 2. The effects of prostaglandins during 24-h chase culture on glucose carbon incorporated into mouse morulae–early blastocysts during a 2-h pulse in medium containing 0.28 mM-[U- 14 C]glucose, 25 mM-lactate and 0.25 mM-pyruvate

Culture conditions and treatment	Labelled glucose carbon (pg-atoms/embryo) present in:			
	Acid-soluble fraction		Acid-insoluble fraction	
	Glycogen	Non-glycogen	Glycogen	Non-glycogen
Pulse 2 h	9.56 $\pm$ 1.37	6.35 $\pm$ 0.69	4.12 $\pm$ 0.30	4.30 $\pm$ 0.37
Chase 24 h				
Control	8.57 $\pm$ 1.63	2.25 $\pm$ 0.25	0.99 $\pm$ 0.11	3.18 $\pm$ 0.35
PGE-2 (1 μ g/ml)	8.32 $\pm$ 1.67	2.45 $\pm$ 0.55	1.12 $\pm$ 0.13	3.50 $\pm$ 0.62
PGE-2 (10 μ g/ml)	8.59 $\pm$ 1.81	2.69 $\pm$ 0.66	0.97 $\pm$ 0.13	3.99 $\pm$ 1.09
PGF-2 α (1 μ g/ml)	7.58 $\pm$ 1.85	1.63 $\pm$ 0.48	0.84 $\pm$ 0.13	2.84 $\pm$ 0.48
PGF-2 α (10 μ g/ml)	8.23 $\pm$ 1.00	2.07 $\pm$ 0.42	1.09 $\pm$ 0.14	3.78 $\pm$ 0.67

Values are means $\pm$ s.e.m. for three replicates.

Table 3. Effects of PGE-2 and PGF-2 α on the production of carbon dioxide and lactate from 0.28 mM-[U- 14 C]glucose by mouse morulae-early blastocysts

Treatment	Carbon dioxide produced	Lactate accumulated
None (control)	3.70 $\pm$ 0.35 (6)	4.22 $\pm$ 0.83 (5)
PGE-2 (1 μ g/ml)	2.65 $\pm$ 0.29 (6)	3.51 $\pm$ 0.78 (5)
PGE-2 (10 μ g/ml)	2.76 $\pm$ 0.40 (6)	4.29 $\pm$ 0.72 (5)
PGF-2 α (1 μ g/ml)	3.17 $\pm$ 0.51 (6)	2.61 $\pm$ 0.06 (5)
PGF-2 α (10 μ g/ml)	2.67 $\pm$ 0.45 (6)	5.00 $\pm$ 1.15 (5)

Values (pmol/embryo h $^{-1}$) are means $\pm$ s.e.m. (number of observations).

these fractions, the non-glycogen acid-insoluble fraction was the least affected with 80% of the label retained after chase compared to 25–35% in the non-glycogen acid-soluble and the acid-insoluble glycogen fractions. However, the presence of PGs during chase culture had no significant effect on degradation of label in any of the biochemical fractions isolated.

The final experiment was carried out to test the influence of PGE-2 and PGF-2 α on the catabolism of glucose by mouse embryos during 4-h incubation (Table 3). The production of CO $_2$ from [U- 14 C]glucose by embryos was significantly inhibited by the presence of prostaglandins in the medium ($P < 0.01$). Both PGE-2 and PGF-2 α reduced carbon dioxide production to a similar extent and appeared to reach their maximum effect at a concentration of 1 μ g/ml, there being no significant dose effect in the statistical analysis. There was much less effect of PGs on lactate production. The addition of PGF-2 α at a concentration of 1 μ g/ml suppressed lactate production ($P < 0.05$). Other treatments did not differ significantly from control.

Discussion

Although data for the concentration of PGs in the uterine fluid of mice do not appear to be available, the concentrations used in the present study might be considered within the physiological range. The levels of PGE-2 and PGF-2 α in uterine tissue during early pregnancy have been reported in the range of 50–100 ng/100 mg tissue (Garg *et al.*, 1979; Phillips & Poyser, 1981). Levels similar to those used in the present experiments have been used in other *in-vitro* investigations (Uehara *et al.*, 1984) and Kirkpatrick (1974) cultured mouse embryos *in vitro* in the presence of PGF-2 α at concentrations up to 100 μ g/ml and noted no adverse effects on embryo development or pregnancy rate after transfer to recipients.

In the present investigations of the effects of PGs on the metabolism of glucose by embryos, only PGE-2 at 10 μ g/ml had an inhibitory effect on incorporation of glucose carbon into acid-soluble glycogen, indicating that embryonic glycogen synthesis is relatively resistant to control by PGs. However, incorporation of [U- 14 C]glucose into the non-glycogen acid-insoluble fraction was stimulated by both PGs, even at 1 μ g/ml. This biochemical fraction represents macromolecules such as proteins, lipids and nucleic acids and this anabolic effect of prostaglandins *in vitro*, if present *in vivo*, may help to prepare the embryo for implantation.

It is difficult to define precisely the mechanism of action of PGs on carbohydrate metabolism. It has been proposed that, at the cellular level, the effects of PGs may be mediated by alterations in intracellular cyclic AMP (cAMP) levels (Kennedy & Armstrong, 1981). Variable and contrary effects of PGs on the carbohydrate metabolism of liver have been reported, including inhibition of glycogenolysis (Wheeler & Epand, 1975) and the activation of the phosphorylase system with the initiation of glycogenolysis (Curnow & Nuttall, 1972).

Whilst PGs had some significant effects on the incorporation of glucose carbon, neither PGF-2 α nor PGE-2, in the concentrations used, had an effect on the turnover of glycogen or other intracellular pools. Therefore, the previously reported increased degradation of glycogen on transfer to ovariectomized mice receiving progesterone (Edirisinghe & Wales, 1984) cannot be attributed to the direct action of these compounds on embryo metabolism. However, an indirect effect of prostaglandins on embryo metabolism through changes in uterine vascular permeability (Kennedy, 1977) and a resultant shift in intrauterine oxygen tension (Mitchell & Yochim, 1968) could offer an explanation for the effects observed.

The experiments undertaken to study the effect of PGs on the rate of CO₂ production indicate that both PGE-2 and PGF-2 α suppress glucose oxidation by preimplantation embryos *in vitro*. However, the rate of aerobic glycolysis measured by the amount of lactate produced was much less affected. PGF-2 α and PGE-2 therefore appear to influence the TCA cycle more than the glycolytic activity of preimplantation embryos.

The findings of the present study demonstrated a direct influence of prostaglandins on the metabolism of glucose by mouse embryos during preimplantation development. However, the results fail to provide an explanation for the increased degradation of acid-soluble glycogen of pulse-labelled embryos *in vivo* under the influence of progesterone (Edirisinghe & Wales, 1984). Factors other than the direct effects of maternal hormones (Wales *et al.*, 1985; Khurana & Wales, 1987) or the prostaglandins E-2 and F-2 α must therefore be responsible for the control of glycogen synthesis and turnover in preimplantation embryos.

We thank Mrs L. McLeod and Ms S. Baker for excellent technical assistance. The work was aided by a grant from the Australian Research Grants Scheme. N.K.K. was supported by a Murdoch University Postgraduate Studentship.

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Received 24 June 1986