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Original article

Activities of cyclooxygenases, and levels of prostaglandins E_2 And $F_{2\alpha}$, in fetopathy associated with experimental diabetic gestation

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Abstract

Aim. – The present study investigated the cyclooxygenase (COX) pathway to elucidate any changes that may be involved in the mechanism(s) underlying diabetic fetopathy.

Methods. – Diabetes was induced in female rats ($n = 12$) by two successive daily injections of 55 mg/kg streptozotocin, while control animals ($n = 10$) were injected with a buffer solution; hyperglycaemia was confirmed by blood glucose levels greater than 11 mmol/L. The study female rats were made pregnant and, on day 15 of gestation, the rats were sacrificed, and the fetuses, placentas and membranes dissected out of the uterine horns. Following morphological examination, the fetuses, placentas and membranes were homogenized, and used to measure COX activities and prostaglandin (PG) E_2 and $PGF_{2\alpha}$ levels.

Results. – Fetuses from diabetic mothers exhibited significantly ($P < 0.05$) shorter crown-to-rump lengths, lower body weights and heavier placental weights. The activity of COX-1 in the fetuses, placentas and membranes from diabetic mothers represented a small percentage of total COX activity compared with that of COX-2. The presence of a COX-1 inhibitor in the control and diabetic rats was investigated and found to be negative. The activity of COX-2 in malformed fetuses from diabetic mothers was significantly lower ($P < 0.05$) compared with non-malformed fetuses from control and diabetic mothers. The mean level of PGE_2 in fetuses from diabetic mothers was significantly ($P < 0.05$) lower than that in controls. In contrast, the biggest increases in $PGF_{2\alpha}$ were observed in the malformed diabetic fetuses, placentas and membranes.

Conclusion. – The increased production of $PGF_{2\alpha}$ probably proceeds, at least in part, independently of the COX pathway and via the isoprostane route. However, it is unclear whether the relatively high levels of $PGF_{2\alpha}$ are causally related to, or simply coincidental with, fetal malformation.

Keywords: Fetopathy; Experimental diabetes; Cyclooxygenases; Prostaglandins

Résumé

Activités des cyclo-oxygénases et concentrations des prostaglandines E_2 and $F_{2\alpha}$ dans la fœtopathie du diabète gestationnel expérimental.

But. – Cette étude avait pour objectif d'étudier l'activité des cyclo-oxygénases (COX) afin de préciser leur place parmi les mécanismes de la fœtopathie diabétique.

Méthodes. – À cette fin, un diabète a été induit chez des rates ($n = 12$) par deux injections journalières successives de 55 mg/kg de streptozotocine. Les rates témoins ont été traitées par une solution tampon. L'hyperglycémie a été confirmée par des glycémies supérieures à 11 mmol/l. Les deux populations de rates, témoins et diabétiques, ont ensuite été fécondées. Au 15^e jour de gestation, les rates ont été sacrifiées. Le fœtus, le placenta et la membrane amniotique de chaque animal ont été enlevés des cornes utérines. Après examen morphologique, le fœtus, leur placenta et les membranes ont été homogénéisés et utilisés pour mesurer les concentrations de COX et de prostaglandines (PG) E_2 et $PGF_{2\alpha}$.

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Résultats. – Les fœtus de rates diabétiques avaient une taille et un poids significativement ($P < 0{,}05$) plus bas que ceux des témoins, le poids du placenta étant significativement ($P < 0{,}05$) plus élevé. L'activité COX-1 représentait un faible pourcentage de l'activité totale des COX comparativement à celle de COX-2. La recherche d'un inhibiteur de COX-1 a donc été réalisée chez les rates témoins et diabétiques et s'est avérée négative. Par ailleurs, l'activité COX-2 des fœtus de mères diabétiques était significativement ($P < 0{,}05$) plus basse que celle des fœtus de mères témoins. Les concentrations moyennes de $PGF_{2\alpha}$ des fœtus de mères diabétiques étaient significativement plus basses ($P < 0{,}05$) à celles des fœtus des rates témoins. A contrario, une augmentation de $PGF_{2\alpha}$ a été observée chez les fœtus diabétiques malformés.

Conclusion. – L'augmentation de la production de $PGF_{2\alpha}$ est probablement au moins en partie produite indépendamment des COX via les isoprostanes. Les concentrations relativement élevées de $PGF_{2\alpha}$ pourraient être en rapport avec les malformations fœtales ou simplement être le fruit du hasard. Cela reste à élucider.

Mots clés : Fœtopathie ; Diabète expérimental ; Cyclo-oxygénases ; Prostaglandines

1. Introduction

Embryonic and fetal life represents the period of development with the greatest number of cell divisions. Diabetes during pregnancy is a recognized medical problem, as maternal and embryonic hyperglycaemia has been reported to cause congenital malformations in diabetic pregnancy [1]. Congenital anomalies among infants of diabetic mothers occur at the rate of 6 to 10%, which is a three- to eightfold increase over rates observed in the non-diabetic population [2,3]. These congenital malformations most commonly involve the central nervous, cardiovascular and skeletal systems [4]. The teratogenic effects of diabetes have been largely attributed to various metabolic factors and, in particular, increased levels of glucose and ketone bodies [5]. In addition, not all diabetic embryopathies are prevented by good glycaemic control, which indicates that factors other than glucose may participate in the process [6]. *S*tudies *in vivo* [7,8] have shown that, in experimentally induced diabetic pregnant rats, dead resorbed embryos and living malformed as well as morphologically non-malformed embryos can be found in the same uterus.

Many factors have been proposed to be important contributors to the mechanism(s) believed to be causative in diabetic embryopathy, including reduced levels of arachidonic acid [9,10], alternations in prostaglandin synthesis [11–13] and increased formation of reactive oxygen species (ROS) [14,15].

Diabetes-induced alteration of prostaglandin E_2 (PGE_2) and arachidonic-acid metabolism have been identified as having teratological capacity [12]. The concentration of PGE_2 is decreased in rat embryos of diabetic mothers, with the lowest values found in malformed embryos and their associated membranes (yolk sacs) between days 9 to 11 of gestation [7]. In addition, cyclooxygenase-2 (COX-2) and PGE_2 [13,16] have been linked to angiogenesis, known to play a pivotal role in shaping the body during organ formation [17].

The present study was undertaken to further investigate the COX pathway in an effort to elucidate the changes that may be involved in the mechanism(s) underlying diabetic fetopathy.

2. Materials and methods

2.1. Experimental animals

Rats from a local Wistar-derived strain with initial weights of 180 ± 20 g were used. The animals were individually housed at a constant humidity and temperature ($20 \pm 5^\circ$ C), with a 12-h light/dark cycle. Tap water and a commercial pellet diet were provided *ad libitum*. All procedures were approved and carried out according to the guidelines of the Animal Ethics Committees of Amman, Applied Science and Alexandria Universities.

2.2. Induction of experimental diabetes

Diabetes was induced in female rats ($n = 12$) as described by El-Bassiouni et al. [7]. This involved two successive daily intraperitoneal injections of 55 mg/kg streptozotocin (STZ; Sigma-Aldrich Chemical Co., Poole, UK) dissolved in sodium-citrate buffer (0.01 M, pH 4.5). Control animals ($n = 10$) were injected with an equivalent volume of the buffer solution. Glucose levels were determined in venous tail blood using a glucometer (Elite). One week after the injections, diabetes was confirmed by a blood glucose level greater than 11 mmol/L.

2.3. Mating

Mating of the diabetic females with healthy non-diabetic males took place overnight. The presence of a sperm mucus plug in the vagina the following morning signified pregnancy (gestational day 0). At the same time, non-diabetic females were similarly mated to serve as controls. Hyperglycaemia in the pregnant rats was confirmed throughout the gestation period by daily measurement of random blood glucose samples from the tail vein.

2.4. Sample collection

Pregnant diabetic and non-diabetic rats were sacrificed by cervical dislocation on gestation day 15. Maternal blood was collected by heart puncture. In each sacrificed rat, the uterus was exposed by cesarean section, and the number of implantations and resorptions recorded. The live fetuses, along with their placentas and membranes, were dissected out of the uterine horns, rinsed carefully in phosphate buffered saline (PBS) and carefully labelled. Overall growth and differentiation of the fetuses were quantified by direct measurement of crown-to-rump length (CRL). The fetuses were examined for their general morphology, and the presence of any disturbed fetal development, such as an open neural tube or growth retardation, and the appearance of specific parts and organs (head, ear, heart, legs,