KINETICS OF PROSTACYCLIN SYNTHETASE IN UMBILICAL ARTERY MICROSOMES FROM NORMAL AND PRE-ECLAMPTIC PREGNANCIES

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- 1 Prostacyclin synthetase in umbilical artery microsomes obeys Michaelis-Menten kinetics.
- 2 The maximum velocity ($V_{\max}$) of prostacyclin synthetase prepared from normal umbilical arteries was significantly higher than the $V_{\max}$ of prostacyclin synthetase in umbilical arteries taken from pre-eclamptic pregnancies.
- 3 The Michaelis-Menten constant (K_m) of the two prostacyclin synthetase preparations was significantly different with the synthetase from pre-eclamptic arteries having a higher affinity for substrate.
- 4 The limiting factor for prostacyclin synthesis is $V_{\max}$ at high substrate concentrations which appears to be the case in umbilical arteries. However, at low substrate concentration there would be no difference in prostacyclin synthesis by the two forms of prostacyclin synthetase.

Introduction

The cause of pre-eclampsia (PET) is as yet undefined. However, recent work has indicated that a deficiency of prostacyclin (PGI_2) may play a significant role in the pathogenesis of PET (Remuzzi *et al.*, 1979, 1980; Terragno & Terragno, 1979; Terragno *et al.*, 1980; Bodzenta *et al.*, 1980; Carreras *et al.*, 1981; Lewis *et al.*, 1981). Prostacyclin is the principle prostaglandin synthesised by blood vessel endothelium, and is a potent vasodilator and inhibitor of platelet aggregation. The clinical symptoms of PET, hypertension, platelet consumption and a decrease in placental perfusion (leading to a slowing of foetal growth) may all be explained by a decrease in PGI_2 levels. It has already been demonstrated that PGI_2 levels in maternal plasma are significantly raised during normal pregnancy (Lewis *et al.*, 1980) and it has been postulated that this increased production contributes to low peripheral vascular resistance seen in foetal circulation (Terragno *et al.*, 1980). We have recently demonstrated that the only prostaglandin-like substance synthesised by microsomal preparation of umbilical arteries is prostacyclin assayed as 6-oxo- $\text{PGF}_{1\alpha}$, the chemical degradation product of PGI_2 (Downing *et al.*, 1981). In the present study we have investigated the kinetics of this preparation comparing umbilical arteries from normal and pre-eclamptic pregnancies.

Methods

Materials

(1- ^{14}C)-arachidonic acid was purchased from the Radiochemical Centre, Amersham (Batch no. 55, specific activity 54.6 $\mu\text{Ci}/\text{mmol}$). Arachidonic acid (grade 1) and reduced glutathione (GSH) were obtained from the Sigma Chemical Company Ltd, hydroquinone was purchased from BDH Chemicals Ltd. Thin-layer chromatography plates (silica gel 60, 0.25 mm thick) were bought from Merck.

The authentic prostaglandin and thromboxane standards were gifts from Dr J. Pike of the Upjohn Company.

Patients

A total of 23 umbilical arteries were studied. Twelve patients were selected as having pregnancy-associated hypertension (PET), with a blood pressure greater than 140/100 mmHg over the week previous to delivery. None of these patients were hypertensive early in pregnancy or postnatally. The normal patients had at no time during pregnancy a blood pressure greater than 120/80 mmHg. No mother had taken any non-steroidal anti-inflammatory drugs within 1 week of delivery. There was no significant difference in the gestational age or in the mode of

delivery between the two groups. There were equal numbers of smokers in the two groups. The mean ($\pm$ s.e. mean) birth weight in the control group was 2.906 ± 0.224 kg and in the PET group 2.65 ± 0.306 kg.

Preparation of microsomal fraction

The umbilical arteries were dissected out of the fresh cord and homogenised in tris acetate buffer (0.1 M, pH 8.0) containing hydroquinone (0.25 mM) and GSH (2 mM) to give a 10% w/v homogenate. A microsomal pellet was obtained by an initial centrifugation at 9000 g for 15 min at 4°C and the supernatant recentrifuged at 100,000 g for 60 min at 4°C. The microsomes were resuspended in fresh buffer. The protein concentration of the microsomes was estimated by the method of Lowry *et al.* (1951).

Radiometric assay

Aliquots (0.2 ml) of the resuspended microsomes were added to 1.8 ml of buffer containing variable concentrations of unlabelled and (1- 14 C) labelled arachidonic acid (between 0.29 and 112 μ M) and then incubated for 4 min at 37°C. The reaction was terminated by acidification with citric acid (2 M) 0.25 ml. Boiled samples were used as controls. The acidified samples were extracted with diethyl ether (2×1.5 vol). The residue of the ethereal extracts was applied to a thin-layer chromatography (t.l.c.) plate accompanied by authentic prostaglandin standards and developed to 15 cm in ethyl acetate/acetone/acetic acid (90 : 10 : 1). After development the standards were visualised using iodine vapour. The chromatogram was divided into 0.5 cm bands which were scraped off. The radioactivity from each band was eluted with 1 ml of methanol and estimated by liquid scintillation counting using Packard 'Insta-Gel' scintillant in a Packard 2650 Tri-Carb liquid scintillation spectrometer. The identity of the 6-oxo-PGF $_{1\alpha}$ peak was verified by gas chromatography/mass spectrometry as previously published (Downing *et al.*, 1981).

Kinetics

For K_m and maximum velocity (V_{max}) determination the substrate concentration was varied between 0.29 and 112 μ M. The value for K_m and V_{max} were calculated using an iterative computer program on a Digital PDP-8/1.

Results

The microsomal preparation of umbilical artery microsomes was found to obey Michaelis-Menten kinetics under the incubation conditions used (Figure

1). The mean $\pm$ s.e. mean V_{max} value for umbilical artery microsomes from normal pregnancies was 1285 ± 168 pmol min $^{-1}$ mg $^{-1}$ protein ($n = 11$), while that for umbilical artery microsomes from PET pregnancies was 707 ± 80 pmol min $^{-1}$ mg $^{-1}$ protein ($n = 12$) (Figure 2). This difference in V_{max} values

Figure 1 The dependence of reaction rate on substrate concentration microsomes of umbilical arteries from normal ($\bullet$) ($n = 5$) and PET pregnancies ($\circ$) ($n = 5$). Mean and s.e. mean values shown.

Figure 2 Distribution of V_{max} values for prostacyclin synthetase in umbilical arteries from normal ($n = 11$) and PET ($n = 12$) pregnancies. The bar indicates the mean, which was 1285 ± 168 pmol min $^{-1}$ mg $^{-1}$ protein and 707 ± 80 pmol min $^{-1}$ mg $^{-1}$ protein respectively ($\pm$ s.e. mean). This difference was highly significant, $P < 0.005$.

proved to be highly significantly different, $P < 0.005$, using a pooled t -test.

The K_m values for prostacyclin synthetase from normal and PET pregnancies (Figure 3) were also found to be significantly different, $P < 0.05$, again using a pooled t -test, the means $\pm$ s.e. mean were $8.53 \pm 1.99 \mu\text{M}$ ($n = 11$) and $3.28 \pm 0.72 \mu\text{M}$ ($n = 12$) respectively.

Figure 3 K_m values for prostacyclin synthetase in microsomes of umbilical arteries from normal ($n = 11$) and PET ($n = 12$) pregnancies. There was a significant difference in the two populations, $P < 0.05$, the respective means ($\pm$ s.e. mean) were $8.53 \pm 1.99 \mu\text{M}$ and $3.28 \pm 0.72 \mu\text{M}$, and are indicated by the bars.

However, the difference in the intrinsic clearance (Figure 4) obtained from:

$$\frac{V_{\max}}{K_m}$$

for the two microsomal preparations was found not to be significant. The value for umbilical arteries from normal pregnancies was $119 \pm 29 \text{ ml min}^{-1} \text{ mg}^{-1}$ protein (mean $\pm$ s.e. mean, $n = 11$) and that for umbilical arteries from PET pregnancies $148 \pm 26 \text{ ml min}^{-1} \text{ mg}^{-1}$ protein (mean $\pm$ s.e. mean, $n = 12$).

Discussion

The $V_{\max}$ values found by this study suggest that umbilical arteries from PET pregnancies are deficient in prostacyclin synthetase when compared to umbilical arteries from normal pregnancies. The K_m data indicates that prostacyclin synthetase in umbilical

Figure 4 The intrinsic clearance for prostacyclin synthetase in microsomes of umbilical arteries from normal ($n = 11$) and PET ($n = 12$) pregnancies. There was no significant difference in the two distributions, the means ($\pm$ s.e. mean) were found to be $119 \pm 29 \text{ ml min}^{-1} \text{ mg}^{-1}$ protein and $148 \pm 26 \text{ ml min}^{-1} \text{ mg}^{-1}$ protein respectively, as indicated by the bars.

arteries from PET pregnancies has a higher affinity for substrate than prostacyclin synthetase found in umbilical arteries from normal pregnancies. There are two possible explanations for this. The first is that there has been a significant modification in the local environment of cyclo-oxygenase enzyme complex. The second that there is a different form of prostacyclin synthetase present in umbilical arteries from PET pregnancies. It is not possible from this study to determine which interpretation is correct.

The values obtained for the intrinsic clearance suggest that at low substrate concentrations (possibly the non-pregnant state) there will be no difference in PGI_2 synthesis between the two types of prostacyclin synthetase. However, the available evidence in the literature (Filshie & Anstey, 1978) indicates that the concentration of free arachidonic acid in umbilical artery tissue is the order of 0.3 mM , a saturating concentration. Thus the determining factor for PGI_2 synthesis is the $V_{\max}$ value, not the substrate concentration. For umbilical arteries from PET pregnancies this would lead to very much lower PGI_2 levels compared to umbilical arteries from normal pregnancies.

This conclusion would be in agreement with four other groups who have now reported that umbilical arteries from severe PET pregnancies produce less

PGI₂-like activity when these arteries are studied using an *in vitro* bioassay (Remuzzi *et al.*, 1980; Bussolino *et al.*, 1980; Carreras *et al.*, 1981; Terragno *et al.*, 1980). Similarly reduced prostacyclin production by placental veins has also been reported (Remuzzi *et al.*, 1980). There is less PGI₂-like activity on bioassay in amniotic fluid from PET pregnancies than in normal pregnancies (Bodzenta *et al.*, 1980). In a recent case report of pregnancy induced hypertension where serial measurements of maternal 6-oxo-PGF_{1α} in plasma were made, there was a dramatic fall in these concentrations with the onset of foetal growth retardation.

In summary the data presented here provides evidence for two forms of prostacyclin synthetase; the form found in umbilical arteries from PET pregnancies has a higher affinity for substrate than

the form found in normal umbilical arteries, but there is less prostacyclin synthetase present in PET umbilical arteries, giving rise to a deficiency in PGI₂ synthesis at the high substrate concentration which occurs in the tissue during pregnancy.

The source of the normal increase in circulating prostacyclin in maternal circulation in pregnancy is not clear. Potentially PGI₂ could be synthesised by the uterus, the placenta or by the foetus. Both foetal blood vessels (Remuzzi *et al.*, 1980) and the uterus (Bodzenta *et al.*, 1980) appear to produce less prostacyclin in PET than in normal pregnancies. This argues for a general defect in prostacyclin production which is supported by results from angiotensin infusion tests (Everett *et al.*, 1978).

I.D. was supported by a grant from the National Fund for Research into Crippling Diseases.

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(Received May 29, 1981)