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TRAPPED BLOOD ELEMENTS WITHIN THE DECIDUA OF THE RAT PREGNANT UTERUS GENERATE A LIPOXYGENASE PRODUCT(S) WHICH INHIBITS MYOMETRIAL PROSTACYCLIN SYNTHESIS

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1 Prostacyclin (PGI_2) production by chopped segments of rat pregnant uterus was low compared with synthesis by separated myometrial tissue. Incubation of separated myometrium with decidua (2:1 by weight) led to an inhibition of myometrial PGI_2 output.

2 Boiling decidual tissue abolished the inhibitory influence on myometrial PGI_2 output. Pre-incubation of decidua with 5,8,11,14-eicosatetraynoic acid (ETA) (30 μg/ml) also suppressed decidual inhibitory activity but indomethacin (30 μg/ml) was ineffective.

3 Incubation of decidual and myometrial tissue with arachidonic acid (AA) 10 μg/ml did not increase the inhibition of myometrial PGI_2 synthesis, even if the decidua were pre-incubated with indomethacin.

4 Myometrial PGI_2 production was reduced if the chopped tissue was pre-incubated with soya bean lipoxidase for 10 min at 4°C. This reduction was reversed if the lipoxidase was incubated with ETA (30 μg/ml) for 30 min at 37°C before addition to the myometrial tissue.

5 Perfusion of the uterus to remove blood elements removed the inhibitory action that the decidua exerted upon myometrial PGI_2 production. PGI_2 synthesis by separated decidual and whole uterine tissue was markedly elevated.

6 The addition of rat blood platelets (0.75×10^9/ml) to incubations of perfused decidual tissue reduced PGI_2 output and restored the inhibitory action that the decidua exerted on myometrial PGI_2 synthesis.

7 It is concluded that a lipoxygenase enzyme contained in blood platelets trapped within the decidual vasculature produces a hydroperoxy acid which inhibits decidual PGI_2 production or myometrial PGI_2 synthesis when the tissues are incubated together. It is suggested that perfusion is a pre-requisite before study of PGI_2 synthesis in highly vascularised tissues.

8 The pathophysiological importance of such platelet lipoxygenase products is discussed.

Introduction

Previous studies have revealed interesting spatial differences in arachidonic acid metabolism by the rat pregnant uterus. Separated decidual tissue has been shown to generate much greater quantities of prostaglandin F (PGF)-like material than the myometrium (Williams, Sneddon & Harney, 1974). Decidual microsomes produced several arachidonic acid metabolites, the chief one being PGE_2 whereas the major product of the myometrial preparations was 6-oxo-$PGF_{1\alpha}$ (Downing & Williams, 1977) which is the hydrolysis product of the unstable prostacyclin (PGI_2) (Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whittaker, Bunting, Salmon, Moncada & Vane, 1976). Direct estimation showed that prostacyclin was produced in much larger quantities by the myometrium than the decidua (Williams, Dembinska-Kiec, Zmuda & Gryglewski, 1978) despite the higher overall prostaglandin synthesizing capacity in decidual tissue.

Moncada, Herman, Higgs & Vane (1977) suggested that prostaglandin cyclic endoperoxides produced by blood platelets may be utilised by the walls of blood vessels for conversion to prostacyclin. This finding raised the possibility that a similar mechanism may operate within the uterus where high cyclooxygenase activity within the decidua may produce cyclic endoperoxides which could be converted to prostacyclin within the myometrium, thereby 'boosting' its inherent synthetic capacity. This theory was tested by comparing prostacyclin production by segments of whole uterus with synthesis by separated decidual and myometrial tissue. A preliminary account of the results was presented at the joint meeting of the Italian and British Pharmacological Societies (El Tahir & Williams, 1981).

Methods

Female Wistar rats were killed on day 22 of pregnancy (day of delivery); the uteri were removed and

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treated as described previously (El Tahir & Williams, 1980; Williams & El Tahir, 1980). Apart from separated fractions of decidua and myometrium, segments of whole uterine tissue were also used.

In other experiments, day 22 pregnant rats were anaesthetized with pentobarbitone (70 mg/kg s.c.). The uterine horns were exposed by laparotomy and displaced onto beds of tissue soaked in warm Krebs solution. The aorta was cannulated retrogradely below the renal arteries and the arterial supply to one uterine horn clamped (control horn). Perfusion of the other horn with Krebs solution at 20 ml/min was started and the vena cava cut to allow the perfusate to escape. Perfusion was continued for 10 min. Uteri were then dissected out and after removal of foetuses and placentae any areas of uterus still containing blood elements were cut out and discarded. Separated myometrial and decidual fractions were then prepared from the perfused and non-perfused horns.

Incubation of samples

Samples of uterine tissue were suspended in Krebs solution (25% (w/v)) and incubated as described previously (El Tahir & Williams, 1980). In many instances tissue samples were pre-incubated with drugs, separated decidual samples were pre-incubated with indomethacin or 5,8,11,14-eicosatetraynoic acid (ETA) for 10 min at 37°C. Arachidonic acid (AA) or rat blood platelets were added immediately after chopping and before starting routine incubation. In some cases decidual tissue was boiled for 10 min before incubation. Separated myometrial tissue was chopped and pre-incubated with or without (control) soya bean lipoxidase for 10 min at 4°C, the sample was then warmed to 20°C and incubated as normal. In some experiments the lipoxidase enzyme was boiled for 10 min or incubated with ETA for 30 min at 37°C before cooling to 4°C for pre-incubation with myometrial tissue. Rat blood platelets or AA were added to the chopped myometrial samples immediately prior to incubation. Allowances for carry-over effects of drugs from decidual to myometrial incubations and/or to platelet samples in aggregation studies were made by ensuring similar drug concentrations were present in control myometrial incubation experiments or in platelet samples.

Assay of prostacyclin in incubation media

The protocol was similar to that used in previous experiments (El Tahir & Williams, 1980).

Separation of rat blood platelets

Pregnant (22 day) rats were anaesthetized with ether and 9 ml of blood withdrawn by cardiac puncture into a syringe containing 1 ml of 3.8% (w/v) sodium citrate. The blood was centrifuged at 200 *g* for 10 min and the platelet-rich plasma (PRP) aspirated and centrifuged at 400 *g* for 30 min. The platelet-poor plasma (PPP) was discarded and the platelet pellet was resuspended in 0.5 ml of 0.9% w/v NaCl solution (saline) by vortex mixing for 30 s. The platelet concentration was determined with a Thrombocounter (Coulter-Instruments). Aliquots of the platelets were added to incubation media to give a final concentration of 0.75×10^9 platelets/ml.

Drugs

The following drugs were used: adenosine 5'-pyrophosphate (ADP), arachidonic acid (grade 1 — porcine liver), soya bean lipoxidase (Type 1, Sigma Chemicals), 5,8,11,14-eicosatetraynoic acid (ETA Roche), indomethacin (Merck, Sharp & Dohme), prostacyclin (PGI_2, Wellcome).

Preparation and storage of substances were as described previously (El Tahir & Williams, 1980).

Results

Prostacyclin synthesis by uterine fractions

Initially, experiments were carried out to compare prostacyclin production by separated myometrial and decidual tissue with that by intact uterine seg-

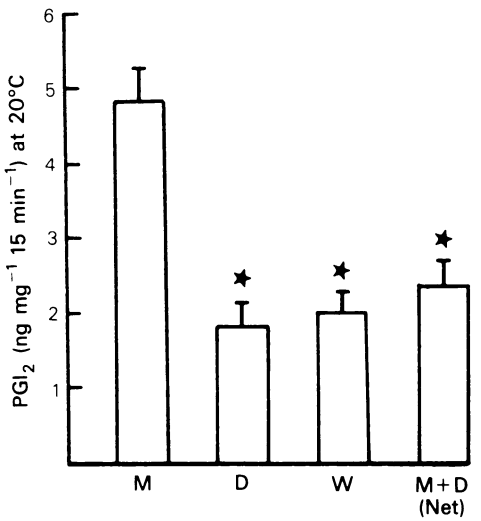

Figure 1 Comparison of prostacyclin synthesis by different uterine fractions. Production was highest in separated myometrial tissue (M) but lower in decidua (D) and in samples of whole uterus (W). Net myometrial prostacyclin generation was reduced when myometrium was incubated with decidua (M + D) 2:1 (w/w). Each column shows mean synthesis with the vertical lines indicating s.e., 7 experiments. Production differing significantly from that of the myometrium is indicated (*$P < 0.005$).

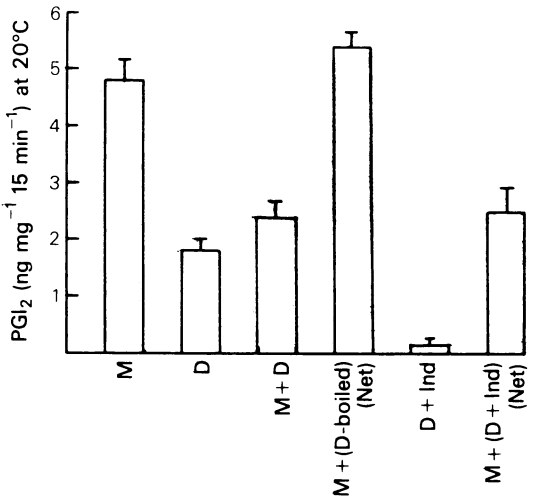

Figure 2 The inhibitory effect which decidual tissue exerts upon myometrial prostacyclin synthesis is abolished by boiling but unaffected by indomethacin. Myometrial (M), decidual (D) and net myometrial prostacyclin generation when incubated with decidua (M + D) were estimated. The inhibitory action of the decidua was eliminated by boiling (M + (D-boiled)) prior to incubation. Incubation with indomethacin (Ind) (30 μg/ml) reduced prostacyclin formation by separated decidua (D + Ind) but when samples of treated decidua were incubated with myometrium (M + (D + Ind)) net synthesis was similar to that seen in the absence of indomethacin. Each column represents mean production from 6 experiments and the vertical lines s.e.

ments (Figure 1). Greatest prostacyclin generation was observed in separated myometrium (4.75 ± 0.39 ng/mg; mean ± s.e.mean; $n = 7$). Decidual samples produced 1.82 ± 0.24 ng/mg and synthesis by whole uterus was similar at 2.01 ± 0.24 ng/mg.

In 7 experiments portions of intact uterus were weighed and after separations of the decidual tissue, the myometrial fraction was reweighed and decidual weight calculated by difference. From these results the myometrium was found to constitute $65 \pm 6\%$ of total uterine weight and the decidua $35 \pm 5\%$. This represents an approximate tissue ratio of 2 parts myometrium to 1 part decidua. The effect of incubating together separated myometrium and decidua in this ratio was to reduce net myometrial prostacyclin generation to 2.31 ± 0.31 ng/mg; this inhibition was significant ($P < 0.005$).

Effects of boiling and pre-incubation of decidua with indomethacin

Experiments were then conducted in which the decidual tissue was exposed to indomethacin before incubation with myometrial tissue (Figure 2). Myometrial and decidual fractions produced 4.75 ± 0.35 and 1.75 ± 0.21 ng/mg of prostacyclin respectively. Pre-incubation of decidua with indomethacin reduced prostacyclin synthesis to 0.15 ± 0.05 ng/mg. Indomethacin addition to myometrium immediately before chopping and incubation does not reduce prostacyclin production (El Tahir & Williams, 1980). Incubation of myometrium with decidua reduced net prostacyclin generation to 2.40 ± 0.32 ng/mg and a similar synthesis of 2.50 ± 0.40 ng/mg occurred if the decidua was exposed to indomethacin. However, when the myometrium was incubated with boiled decidua, prostacyclin production increased to 5.40 ± 0.28 ng/mg a value not significantly different from that for control myometrial incubations.

Eicosatetraynoic acid (ETA)

Similar experiments were then carried out using ETA (Figure 3). Pre-incubation of decidual tissue with ETA 30 μg/ml reduced prostacyclin synthesis from 1.65 ± 0.18 to 0.25 ± 0.08 ng/mg ($n = 6$). Net production by myometrium incubated with decidua was 2.50 ± 0.24 ng/mg which rose to 4.50 ± 0.46 ng/mg if the decidua was pre-incubated with ETA. This synthesis was similar to that seen in control myometrial samples.

Arachidonic acid

When myometrial tissue was incubated in the presence of AA, prostacyclin production was markedly stimulated to 7.20 ± 0.62 ng/mg ($n = 6$). However, decidual generation at 2.00 ± 0.20 ng/mg in the presence of AA was similar to that in the absence of added precursor. Incubation of myometrium plus

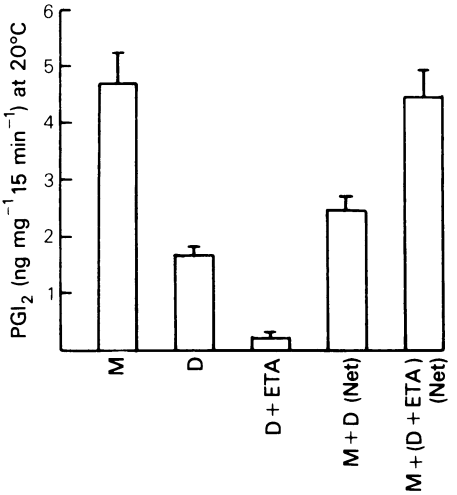

Figure 3 The effect of eicosatetraynoic acid (ETA) upon decidual inhibition of myometrial prostacyclin synthesis. Production by separated myometrium (M) was consistently higher than in decidual tissue (D). Decidual prostacyclin production was reduced after incubation with ETA (D + ETA). The inhibitory effect of the decidua on myometrial prostacyclin production (M + D) was reversed if the decidua had been pretreated with ETA (M + (D + ETA)). Each column represents the mean of 6 experiments and the vertical lines s.e.

decidua in the presence of AA reduced net myometrial prostacyclin synthesis to 4.01 ± 0.32 ng/mg, a 45% reduction (a similar 40% inhibition was noted in the absence of AA i.e. in control incubations). The net production of prostacyclin by myometrium incubated with indomethacin-treated decidua and AA was similar at 3.70 ± 0.15 ng/mg.

Soya bean lipoxidase

Myometrial prostacyclin output was also reduced if after chopping it was pre-incubated with soya bean lipoxidase at 4°C (Figure 4a). Depression of synthesis was dose-related and significant inhibition was seen at a dose of 1 mg/ml ($P < 0.02$; $n = 7$). If the enzyme was boiled before incubation with myometrial tissue then no inhibition of prostacyclin synthesis was noted.

The effect of ETA on the lipoxidase-induced inhibition of myometrial prostacyclin production is shown in Figure 4b. Incubation of myometrium with lipoxidase (1 mg/ml) reduced prostacyclin output from 4.05 ± 0.23 ng/mg to 2.37 ± 0.08 ng/mg ($P < 0.02$; $n = 5$). However, if the lipoxidase was pre-incubated with ETA (30 μg/ml) before addition to the myometrium then prostacyclin output increased to 3.44 ± 0.29 ng/ml. This was not significantly different from production by control myometrial samples.

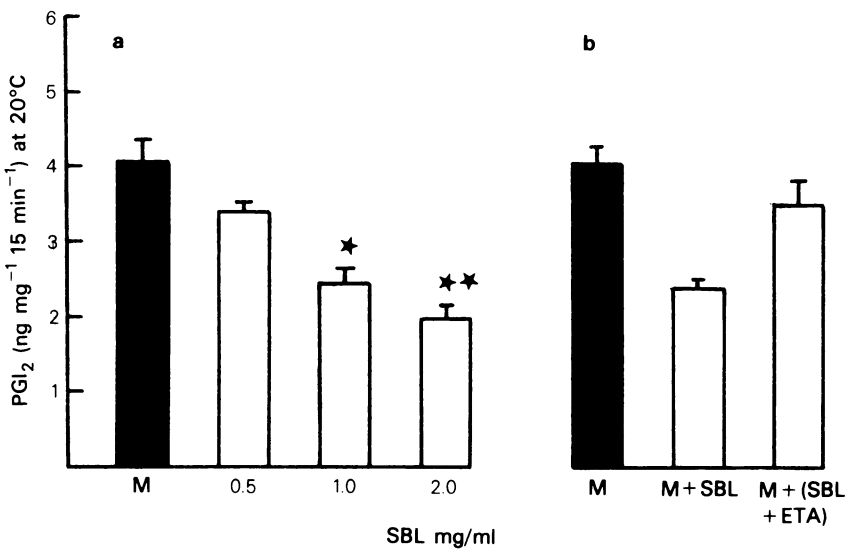

Figure 4 The inhibitory effect of soya bean lipoxidase (SBL) on myometrial prostacyclin production and its reversal by eicosatetraynoic acid (ETA): (a) shows the mean basal prostacyclin release from the myometrium (solid column) and output in the presence of increasing doses of SBL (open columns). Asterisks indicate significant differences (*$P < 0.02$; **$P < 0.005$) from basal synthesis. (b) Shows basal myometrial prostacyclin synthesis (M) which is reduced on incubation with SBL 1 mg/ml (M + SBL). This inhibition was reversed by preincubation of SBL with ETA (M + (SBL + ETA)). Vertical lines represent s.e. from 7 experiments (a) and 5 experiments (b).

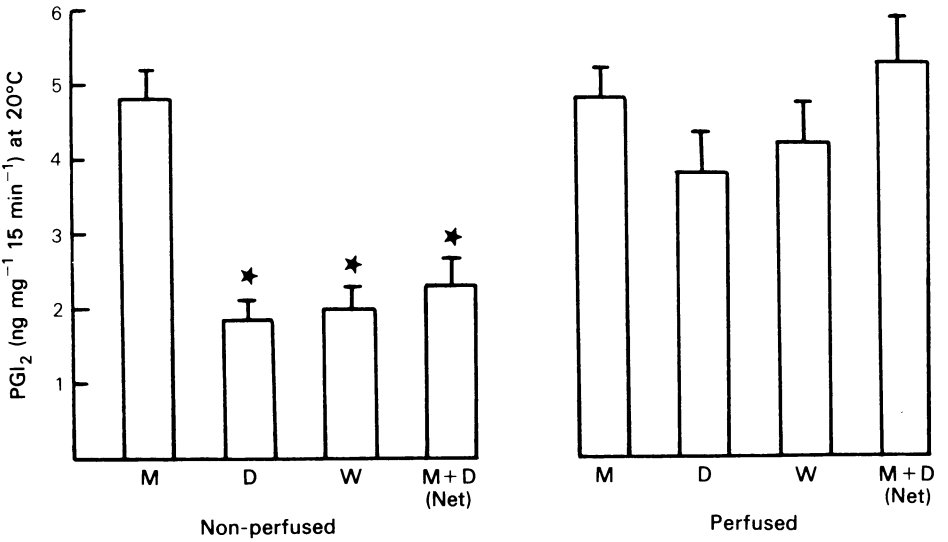

Figure 5 Comparison of prostacyclin synthesis by different uterine fractions from non-perfused and perfused uterine horns. Production by non-perfused fractions of myometrium (M), decidua (D), whole uterus (W) and net prostacyclin production by myometrium incubated with decidua (M + D) were assessed. Prostacyclin synthesis was significantly lower in other fractions than in the myometrium (*$P < 0.005$). In the perfused preparations (prepared from the contralateral uterine horns), prostacyclin generation by the perfused decidual and whole uterine fractions increased markedly and decidual tissue no longer exerted an inhibitory effect on myometrial prostacyclin output. Vertical lines represent s.e.; 7 experiments.

Uterine perfusion

A comparison of the prostacyclin synthesis by different uterine fractions prepared from the perfused and non-perfused (control) horns of pregnant rat uteri is shown in Figure 5. Production by non-perfused fractions was similar to the values shown already in Figure 1. However, perfusion caused several marked changes in prostacyclin generation. Firstly, decidual production was increased to 3.73 ± 0.55 ng/mg ($n = 7$) from 1.82 ± 0.24 ng/mg in the non-perfused samples, a significant difference ($P < 0.05$). Similarly, synthesis by whole uterine fractions increased from 2.01 ± 0.24 ng/mg (non-perfused) to 4.21 ± 0.52 ng/mg after perfusion ($P < 0.02$). Perfused decidua when incubated with myometrial tissue no longer inhibited prostacyclin production which reached 5.25 ± 0.62 ng/mg compared to 2.31 ± 0.31 ng/mg with non-perfused decidua. Myometrial prostacyclin production was the only case in which perfusion did not effect an increase. After perfusion the prostacyclin output by the different fractions was similar.

Blood platelets

The ability of rat blood platelets to restore inhibitory activity to perfused decidua was then investigated (Figure 6). Incubation of perfused decidua with rat blood platelets (0.75×10^9/ml) reduced prostacyclin

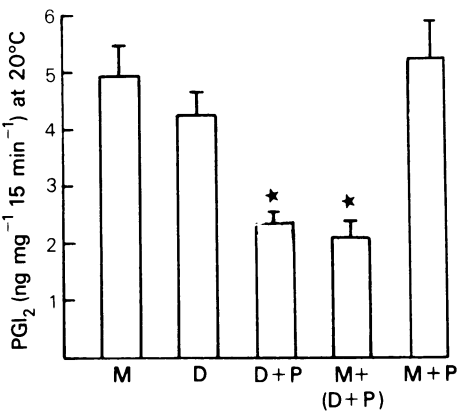

Figure 6 Rat blood platelets inhibit prostacyclin formation when incubated with perfused uterine tissue fractions. Decidual tissue (D) when incubated with rat blood platelets (D + P) produced significantly less prostacyclin than control samples and also reduced production when incubated together with myometrium (M + (D + P)). Incubation of platelets with myometrial samples (M + P) did not alter production from control values (M). Asterisk denotes significant difference from appropriate control ($P < 0.05$). Vertical lines represent s.e., 3 experiments.

production from 4.26 ± 0.23 ng/mg ($n = 3$) to 2.29 ± 0.18 ng/mg ($P < 0.05$). Conversely, myometrial synthesis increased slightly from 4.96 ± 0.54 ng/mg to 5.28 ± 0.63 ng/mg in the presence of a similar concentration of blood platelets although this increase was not significant. However, when perfused myometrium was incubated with perfused decidua plus platelets the prostacyclin output was reduced to 2.07 ± 0.23 ng/mg ($P < 0.05$). This production was similar to that seen when the myometrium was incubated with non-perfused decidua.

Discussion

The present experiments clearly show that the separated decidual tissue fraction of the rat pregnant uterus synthesizes lesser amounts of prostacyclin than the myometrium in accordance with earlier studies (Williams *et al.*, 1978; Williams & El Tahir, 1980). This difference was originally attributed to differences in the concentrations of prostacyclin synthetase within these tissues (Williams *et al.*, 1978). However, the finding that segments of whole uterus also produced much less prostacyclin than the myometrium alone suggested an inhibitory factor may be involved (synthesis was below that to be expected from the fact that approximately one third of the tissue weight is decidua producing less prostacyclin than the myometrium). This theory was supported by the finding that incubation of myometrium in the presence of decidua significantly reduced myometrial prostacyclin production and confirmed the decidua as the origin of the inhibition. Boiling was found to destroy decidual inhibitory activity indicating that the factor or the system producing it (perhaps an enzyme) was heat labile. Assuming the factor was an enzyme product, attempts were made to identify the enzyme by the use of inhibitors. The inability of indomethacin to block production of the inhibitory factor suggested that it was not a cyclo-oxygenase product. However, ETA effectively abolished production of the decidual inhibitor. Although this drug is a cyclo-oxygenase inhibitor it also exerts a lipoxygenase-blocking action (Ahern & Downing, 1970; Downing, Ahern & Bachta, 1970). This enzyme can convert unsaturated fatty acids to lipid hydroperoxides, several of which are potent inhibitors of prostacyclin synthetase (Salmon, Smith, Flower, Moncade & Vane, 1978). The fact that the inhibitory factor is probably a lipoxygenase product would also explain the ineffectiveness of indomethacin in blocking its synthesis as this drug does not inhibit lipoxygenase at doses which block cyclo-oxygenase (Hamberg & Samuelsson, 1974).

As the inhibitory factor appeared to be an AA metabolite the influence of exogenous AA on inhibitor production was examined. Myometrial prostacyclin was increased by arachidonate as reported previously (El Tahir & Williams, 1980) and was inhibited when incubated with decidua plus precursor. However, the % inhibition was similar to that noted in the absence of AA. In an attempt to increase synthesis of inhibitor, decidual tissue was pre-incubated with indomethacin before AA addition, the principle being that after cyclo-oxygenase inhibition, more AA is converted by the lipoxygenase to hydroperoxy acids (Hamberg, 1976). However, indomethacin failed to increase inhibitor production. One possible reason for lack of effect of indomethacin and excess arachidonate could be that excess endogenous substrate is available for conversion in the decidua. This is supported by the finding that added arachidonate did not increase decidual prostacyclin production.

Inhibition of myometrial prostacyclin production could be achieved not only by incubation with decidual tissue but also with the plant enzyme, soya bean lipoxidase. This inhibition was probably due to the lipoxidase converting myometrial arachidonate to 15-hydroperoxy arachidonic acid (Hamberg & Samulesson, 1967) which is known to inhibit prostacyclin synthesis in the myometrium (El Tahir & Williams, 1980). The inhibitory effect of the lipoxidase was abolished by boiling or by incubation with ETA as was that of the decidua. Thus the experiments indicated that the decidua contained a lipoxygenase enzyme producing an inhibitory product although no information had been obtained as to the location of the enzyme within the decidual tissue. The decidua is highly vascularised and as blood platelets have been shown to contain high lipoxygenase activity (Hamberg & Samuelsson, 1974; Nugteren, 1975), trapped platelets were considered as a possible source of the inhibitory activity. Thus uterine horns were perfused until visibly free of blood elements and prostacyclin production by different uterine fractions compared with non-perfused samples. Decidual prostacyclin synthesis in the perfused tissue was significantly greater than in the non-perfused tissue indicating that the inhibitory factor arising from the blood elements directly suppresses decidual prostacyclin synthesis. Decidual samples no longer inhibited myometrial prostacyclin production, again indicating that perfusion had removed the inhibitory factor. In fact, myometrial synthesis was increased slightly in the presence of perfused decidua, perhaps indicating that the myometrium can utilize precursor from the decidua (recent experiments suggest a similar mechanism may operate in the non-pregnant human uterus (Abel & Kelly, 1979)). Control myometrial prostacyclin production was unaffected by perfusion so presumably only a small proportion of blood elements are trapped in this tissue.

The platelets were confirmed as a source of inhibitory material by incubating decidua with separated rat

blood platelets; prostacyclin synthesis was reduced to levels similar to those synthesized by non-perfused decidual samples. Furthermore platelet addition restored the inhibitory effect. Surprisingly, incubation of myometrium alone with blood platelets did not reduce prostacyclin output (in contrast to soya bean lipoxidase). Two explanations for this discrepancy are possible; firstly a co-factor present in the decidual tissue may be needed for the activation of the platelet lipoxygenase. Indeed the production of 12-L-hydroxy eicosatetraenoic acid has been shown to be stimulated by calcium ions (Deykin, Russel & Vaillancourt, 1979) and phytohaemagglutinin (Parker, Stenson, Huber & Kelly, 1979). Perhaps a more plausible theory is that AA within the decidua is more readily available to the platelet lipoxygenase than substrate in the myometrium. This could simply be due to the fact that the decidua has more substrate; certainly the decidua has a higher prostaglandin synthesizing capacity in the absence of added precursor than the myometrium (Williams *et al.*, 1974). The fact that platelets do not inhibit myometrial prostacyclin production also suggests that decidual inhibition is due to the formation of an inhibitor and not simply due to the lipoxygenase 'stealing' myometrial substrate.

The concentration of rat blood platelets used in these experiments was higher than would be found in the blood and other blood-borne sources of lipoxygenase may be involved. Both neutrophils and lymphocytes contain a lipoxygenase enzyme (Borgeat, Hamberg & Samuelsson, 1976; Parker *et al.*, 1979). Neutrophil involvement is unlikely however, as the lipoxygenase is not susceptible to inhibition by ETA (Borgeat *et al.*, 1976). Thus the blood platelets are probably the major source of lipoxygenase products in the non-perfused decidua although we have noted that the perfused decidua still has a residual capacity to convert radiolabelled AA to hydroxy acid products (El Tahir & Williams, unpublished).

The experiments described indicate that trapped blood elements (probably platelets) within decidual tissue can markedly reduce prostacyclin output not only in the decidua but also in the tissue to which it is intimately connected, the myometrium. This suggests that in any study involving measurement of prostacyclin synthesis in a tissue, it is essential to determine whether production is altered after perfusion. If prostacyclin generation increases, then perfusion should be adopted routinely. The present results have also meant that the capability of the rat pregnant decidua to produce prostacyclin has had to be reassessed as the published data using non-perfused tissue are underestimates of the true synthetic capability (Williams *et al.*, 1978).

It is also pertinent to consider whether the inhibition of prostacyclin output by blood elements could have a pathophysiological role. Prostacyclin production by the pregnant uterus and placenta has been considered as contributing a vasodilator effect to the utero-placental circulation (Myatt & Elder, 1977; Williams *et al.*, 1978). Pregnancy hypertension (pre-eclampsia) is associated with thrombocytopenia (Bonnar, 1973). This may be due to increased platelet deposition in the placental vasculature, which is known to occur in pregnancy (Sheppard & Bonnar, 1974). Perhaps platelet-derived lipoxygenase products inhibit vascular prostacyclin synthesis with a resulting vasoconstriction and rise in blood pressure. On a wider basis, atherosclerotic blood vessels produce less prostacyclin than normal vessels (Gryglewski, Dembinska-Kiec, Zmuda & Gryglewska, 1978; Sinzinger, Feigel & Silberbauer, 1979). As atherosclerotic plaques contain many platelet deposits (Woolf, 1978) hydroperoxy acids produced by the platelet lipoxygenase could contribute to this depressed prostacyclin production, adding yet another facet to the role of arachidonic acid metabolism in thromboembolic disease.

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