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FACTORS AFFECTING PROSTACYCLIN FORMATION BY THE RAT PREGNANT MYOMETRIUM

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1 The scraped myometrium of the pregnant uterus of the rat, when chopped and incubated, released an antiaggregatory material closely resembling prostacyclin (PGI_2). The material was conclusively identified as PGI_2 by identification of its hydrolysis product 6-oxo-prostaglandin $F_{1\alpha}$ (6-oxo-$PGF_{1\alpha}$) by gas chromatography-mass spectrometry (GC-MS).

2 Peak concentrations of PGI_2 were detected after 15 min incubation at 20°C. These concentrations were significantly higher than those detected at 37°C, when largest amounts of prostacyclin were formed after 3 min incubation. The concentrations of 6-oxo-$PGF_{1\alpha}$ detected were similar at the different temperatures.

3 When samples were incubated at pH 8 and 20°C, peak concentrations of prostacyclin were maintained between 15 and 35 min of incubation. When pH 7.4 was employed, prostacyclin concentration in the incubate fell to undetectable limits within this time.

4 Incubation of the chopped myometrium with arachidonic acid or phospholipase A_2 stimulated prostacyclin production.

5 Preincubation of myometrial tissue for 10 min at 37°C with inhibitory drugs before chopping reduced prostacyclin output. The doses needed to reduce PGI_2 output by 50% (ID_{50}) were: mepacrine (280 μg/ml), indomethacin (20 μg/ml), 5,8,11,14 eicosatetraynoic acid (23 μg/ml), 15-hydroperoxy arachidonic acid (23 μg/ml) and tranylcypromine (225 μg/ml).

6 It is suggested that due to the large amounts of material available, the rat pregnant myometrium is a useful model for the study of factors affecting prostacyclin synthesis.

Introduction

In 1976 Moncada, Gryglewski, Bunting & Vane showed that prostaglandin endoperoxides were converted by microsomal fractions of blood vessels into an unstable unidentified substance that had potent vasodilator and platelet anti-aggregatory activities. This new metabolite was successfully isolated and its structure identified; it was called prostacyclin or PGI_2 (Johnson, Morton, Kinner, Gorman, McGuire, Sun, Whittaker, Bunting, Moncada & Vane, 1976). As rapid and selective assays for measurement of prostacyclin became available e.g. relaxation of spiral strips of bovine coronary artery (Kulkarni, Roberts & Needleman, 1976) or the inhibition of induced-platelet aggregation (Moncada *et al.*, 1976) a number of papers were published showing prostacyclin was synthesized in many different tissues (for review see Moncada & Vane, 1978). Experiments in our laboratory indicated that in the pregnant rat uterus, prostacyclin was the major arachidonic acid metabolite produced by the myometrial tissue. For instance myometrial microsomes converted radiolabelled arachidonic acid to a substance that had chromatographic properties identical to 6-oxo-$PGF_{1\alpha}$ in several solvent systems (Downing & Williams, 1977). Furthermore, samples of chopped myometrial tissue generated a substance that had parallel activity to authentic prostacyclin in relaxing strips of bovine coronary artery and in inhibiting adenosine 5'-pyrophosphate (ADP)-induced platelet aggregation (Williams, Dembinska-Kiec, Zmuda & Gryglewski, 1978; Williams & El Tahir, 1980a). The rat pregnant uterus thus provided an ideal tissue for studying effects of different incubation conditions and drugs upon prostacyclin formation.

Methods

Female Wistar rats were mated overnight and the following day the presence of a cervical plug was taken as evidence of fertilization. This day was denoted day

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l of pregnancy. Animals were killed on selected days of pregnancy. The abdomen was opened and the 2 uterine horns transferred to ice-cold Krebs solution. After removal of the uterine contents the myometrial areas which lay beneath the placentae were carefully dissected out and discarded as they do not produce prostacyclin (Williams & El Tahir, 1980a). Decidual tissue was then separated from the myometrium by scraping with a microscope slide (Williams, 1973) and discarded. Myometrial tissue was then divided into fractions of known weight (approx. 200 to 1000 mg) and placed in ice-cold Krebs solution until required. Samples were blotted dry and then suspended in ungassed Krebs solution (pH 8) or Tris buffer (pH 7.4 or pH 8) to give a 25% (w/v) mixture. The reaction was initiated by chopping the sample finely with dissecting scissors and incubating at 20°C or 37°C. Substances influencing arachidonic acid metabolism were added after chopping the tissue, or the sample of uterine tissue was pre-incubated with the drug for 10 to 30 min at 20°C or 37°C before chopping. Arachidonic acid or phospholipase A_2 were added to the incubation medium immediately after chopping the myometrium. After the required incubation period, the medium was aspirated, stored on ice and assayed as quickly as possible.

Measurement of platelet aggregation

New Zealand white rabbits were anaesthetized with ether. Blood was collected by cardiac puncture without the use of a syringe into 3.8% sodium citrate (9 vol of blood to 1 vol of citrate). Platelet-rich plasma (PRP) was prepared by centrifuging the blood at 200 *g* for 10 min at 20°C. PRP was aspirated and divided into 0.5 ml aliquots in plastic tubes (Luckham PT/0944). Tubes were placed in an aggregometer (Bryston Ltd.) warmed to 37°C and stirred at 1100 rev/min. The output from the aggregometer was connected to a suitable pen recorder. In each experiment the smallest dose of ADP that produced an irreversible aggregation response lasting at least 4 min was found (5 to 20 μM final concentration in the cuvette) and this dose of ADP was used throughout the experiment. Samples of incubation media or authentic prostacyclin (max 10 μl) were added to the cuvette 1 min before the addition of ADP and the antiaggregatory effect recorded. The prostacyclin content of the sample was then estimated by a 2 + 2 doses assay. The intra- and inter-coefficients of variation for the assay were 5.6 and 6.9% respectively (Williams & El Tahir, 1980b). Statistical significance was calculated by Student's *t* or paired *t* tests.

When incubating myometrial tissue with drugs that influence arachidonic acid metabolism, the possibility that such drugs may influence platelet aggregation has to be considered. To control such carry-over effects, each drug was added (in a volume equal to the largest volume of incubate tested and in a concentration equal to that used for pre-incubation with the myometrium) to each sample of PRP concomitantly with ADP, the only exception being when the drug was pre-incubated with the myometrial tissue sample. When studying the effect of arachidonic acid and phospholipase A_2, the PRP was pretreated with indomethacin 10 μg/ml for 10 min at 37°C. This abolished the aggregatory effects of arachidonic acid without affecting platelet sensitivity to ADP.

Gas chromatographic–mass spectrometric analyses

In some experiments, after incubation of myometrial tissue, incubation fluid was acidified to pH 3 with 2 M citric acid and extracted twice with 2 vols of ether. The combined etheral extracts were evaporated under nitrogen. To each reconstituted residue was added 25 ng of 3,3,4,4-tetradeutero 6-oxo-$PGF_{1\alpha}$ as internal standard. Subsequent purification by XAD-2, extraction, thin-layer chromatography and quantitation by gas chromatography-mass spectrometry (GC-MS) using multiple ion detection have already been described (Hensby, Fitzgerald, Friedman, Lewis & Dollery, 1979).

Drugs

The following drugs were used: adenosine 5′ pyrophosphate (ADP), arachidonic acid (grade 1—porcine liver), quinacrine hydrochloride (mepacrine), phospholipase A_2 (porcine pancreas, Sigma Chemicals), 5,8,11,14-eicosatetraynoic acid (ETA, Roche), indomethacin (Merck, Sharp & Dohme), prostacyclin (PGI_2, Wellcome), prostaglandins D_2, E_2 and $F_{2\alpha}$ (Upjohn) and tranylcypromine sulphate (Smith, Kline & French).

15-Hydroperoxy archidonic acid was prepared by a modification of the method of Funk, Isaac & Porter (1976).

Where possible, substances were dissolved in Krebs solution (pH 8). Indomethacin was solubilised by addition of an equivalent of sodium carbonate. Eicosatetraynoic acid and 15-hydroperoxy arachidonic acid were first dissolved in a minimal amount of 95% (v/v) ethanol. Arachidonic acid (10 mg/ml) was stored in toluene; the solvent was evaporated under nitrogen before dilution. Prostacyclin as the sodium salt was dissolved in 0.1 M Tris buffer (pH 9) to a concentration of 100 μg/ml. Aliquots of this solution were deep frozen. Dilutions were made in Tris buffer or Krebs solution (pH 8) and kept on ice during use.

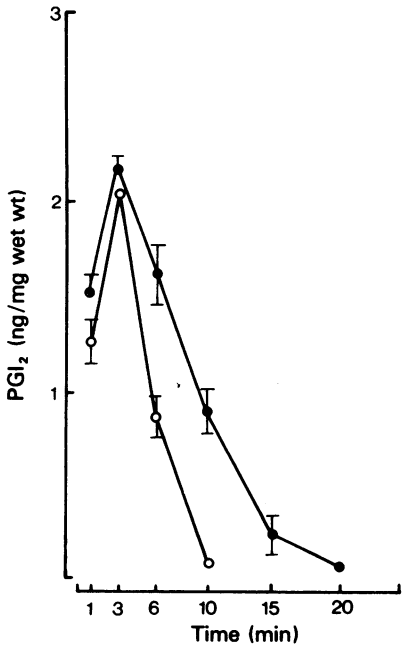

Figure 1 Time course of the release of prostacyclin-like material from the rat pregnant myometrium when incubated at 37°C in Tris-buffer pH 7.4 (○) or pH 8 (●). Each point represents the mean from 4 experiments, vertical lines represent s.e.

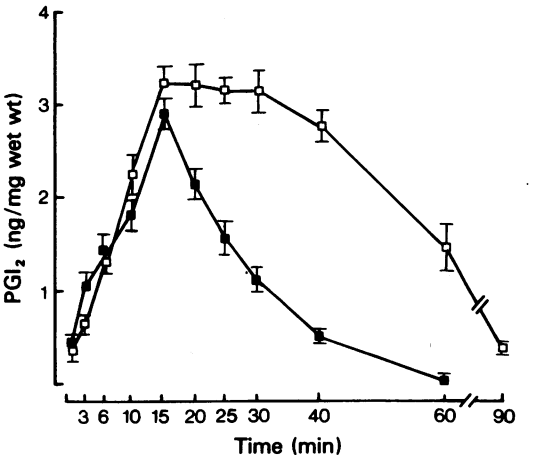

Figure 2 Time course of the release of prostacyclin-like material from the rat pregnant myometrium incubated at 20°C in Tris-buffer pH 7.4 (■) or pH 8 (□). Points represent mean from 4 experiments; vertical lines represent s.e.

Results

Effect of temperature

In an initial series of experiments, samples of myometrial tissue were incubated (in either Tris pH 7.4 or Krebs solution pH 8) for different amounts of time at 37° or 20°C. The release of anti-aggregatory activity expressed as prostacyclin is shown in Figure 1. When incubated at 37°C there was a sharp rise in production of anti-aggregatory material which reached a maximum after 3 min (2.06 ± 0.14 ng/mg; mean ± s.e. mean; $n = 4$). From this point the amount of activity declined and was undetectable after a 10 min incubation period. If samples were incubated at 20°C the generation of prostacyclin-like material rose more slowly attaining a maximum of 2.9 ± 0.32 ng/mg after 15 min incubation. This production was significantly greater than that seen when incubations were carried out at 37°C ($P < 0.05$). Apart from maximal production being greater there was also a much slower decline in peak concentrations; after 25 min incubation the activity detected was still 50% of the maximum value and minimal amounts were not detected until incubation was of 60 min duration. Thus 20°C was chosen as the most suitable incubation temperature.

Determination by GC-MS of the 6-oxo-$PGF_{1\alpha}$ content of a sample taken after 3 min incubation at 37°C was found to be 3.91 ng/mg compared with 2.16 ng/mg prostacyclin equivalents detected by anti-aggregatory activity. After 15 min incubation at 20°C, the 6-oxo-$PGF_{1\alpha}$ production was similar at 4.16 ng/mg. This correlated well with the prostacyclin estimation (4.08 ng/mg). Such close agreement between 6-oxo-$PGF_{1\alpha}$ and prostacyclin values after incubation at 20°C was confirmed in two further experiments using uteri from 19 and 20 day pregnant animals.

Effect of pH

Having determined a suitable temperature for incubation the effects of altering the pH of the incubation medium were determined. The effect of incubating samples of myometrial tissue in Tris buffer at pH 7.4 and pH 8 at 20°C are shown in Figure 2. The pH of the media had no effect on the rate at which peak production of anti-aggregatory activity was attained, this being 15 min. Furthermore, the highest amounts of activity detected in the media at pH 7.4 and pH 8 were not significantly different. The most striking finding was the time course of the decline of the anti-aggregatory material contained in the samples after maximal concentrations were achieved. When incubated at pH 7.4, activity declined from 15 min onwards reaching a minimum after 60 min. However, when incubation was carried out at pH 8 there was no

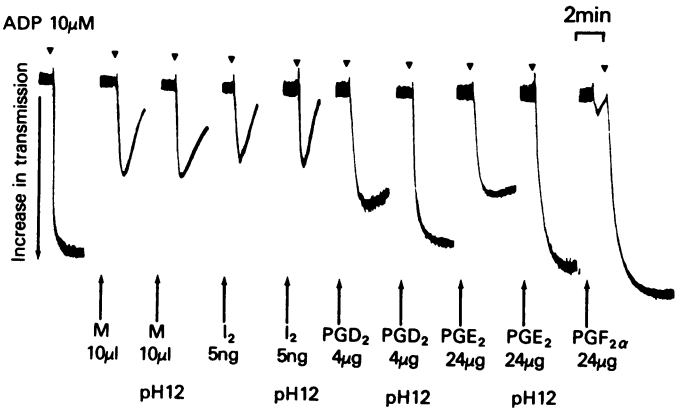

Figure 3 The alkaline stability of the myometrial anti-aggregatory material and authentic prostacyclin. Aggregation of aliquots of rabbit citrated platelet-rich plasma was induced by ADP 10 μM(▼). The anti-aggregatory effects of aliquots of myometrial incubation medium (M); authentic prostacyclin (I_2); prostaglandin D_2 (PGD_2) and PGE_2 were studied before and after maintenance under severe alkaline conditions (pH 12). The activity of the myometrial incubate and prostacyclin were unaffected by alkaline treatment whereas the activity of PGD_2 and PGE_2 were completely abolished by this treatment. $PGF_{2\alpha}$ in large doses was found to initiate a small pro-aggregatory effect.

significant difference in the concentration of anti-aggregatory material in the incubated samples between 15 and 30 min of incubation and even after 60 min of incubation the activity in the incubation medium was still 50% of the maximum value. Thus, pH 8 was chosen as the most suitable pH for subsequent experiments. Tris buffer or Krebs solution did not alter the maximal amounts of anti-aggregatory material which were produced.

As the anti-aggregatory material appeared to be more stable at pH 8 than pH 7.4, we tested its stability under more severe alkaline conditions. The results from one such experiment are shown in Figure 3. The tracing shows the aggregation response induced by 10 μM (final concentration) of ADP in rabbit citrated platelet-rich plasma. The addition of 10 μl of myometrial incubation medium caused a large reduction in the ADP-induced aggregation. An aliquot of this medium was then adjusted to pH 12 and maintained thus for 60 min at 4°C. After acidification to pH 8 the anti-aggregatory activity in the sample was again estimated and it was found to be identical to the sample prior to alkalinization. Authentic prostacyclin at a dose of 5 ng when treated in a similar manner was also found to be stable. The anti-aggregatory effects of other prostaglandins which may be released from the uterus and their alkaline stability were then investigated. As Figure 3 shows, 4 μg of PGD_2 gave only a weak anti-aggregatory effect and on storing at pH 12 this anti-aggregatory activity was completely abolished. PGE_2 at a dose of 24 μg exhibited some anti-aggregatory effects but again these were abolished on alkalinization. At a dose of 24 μg $PGF_{2\alpha}$ itself exhibited a small pro-aggregatory effect and potentiated the aggregatory response to ADP.

Effects of phospholipase A_2 and arachidonic acid

In a series of experiments samples of myometrial tissue were incubated at 20°C in the presence of different concentrations of phospholipase A_2 (Figure 4). Release by control fractions of myometrium was found to be 3.04 ± 0.24 ng/mg ($n = 4$) and this increase in the presence of 0.5 u/ml of phospholipase A_2 to 6.14 ± 0.43 ng/mg; a 102% increase. There was a greater increase in the presence of 1 u/ml of phospholipase A_2 to 8.4 ± 1.11 ng/mg. These are both significant increases ($P < 0.01$; $n = 4$). In a second series of experiments using 22-day pregnant myometrium, the release by control fractions was found to be 4.32 ± 0.45 and this increased to 6.5 ± 0.52 ng/mg ($n = 4$), a 50% increase. There was a further increase in presence of phospholipase A_2 1 u/ml to 8.50 ± 0.94 ng/mg. Both these values are significant increases ($P < 0.05$).

In several experiments the effects of incubating the myometrium in the presence of different concentrations of arachidonic acid were studied (Figure 5). Myometrial tissue from day 22 pregnant rats was used and the basal release was found to be 4.07 ± 0.43 ng/mg ($n = 5$). A graded stimulation in output of prostacyclin-like material was noted. In the presence of 5 μg/ml of arachidonic acid, the prostacyclin production had increased to 6.62 ± 0.67 ng/mg

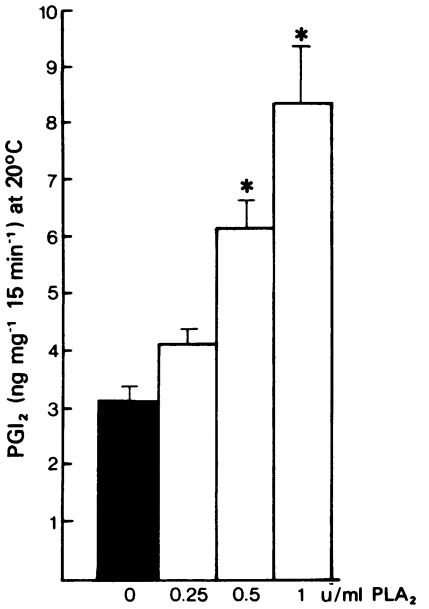

Figure 4 The stimulant effect of phospholipase A_2 (PLA_2) on the release of prostacyclin from the rat pregnant myometrium (day 20 of pregnancy). The mean basal release of prostacyclin from the myometrium (solid column) or stimulated release in the presence of increasing doses of PLA_2 (open columns) in 4 experiments is shown. Vertical lines represent s.e. Asterisks show significant differences ($P < 0.01$) between basal release and PLA_2-treated samples.

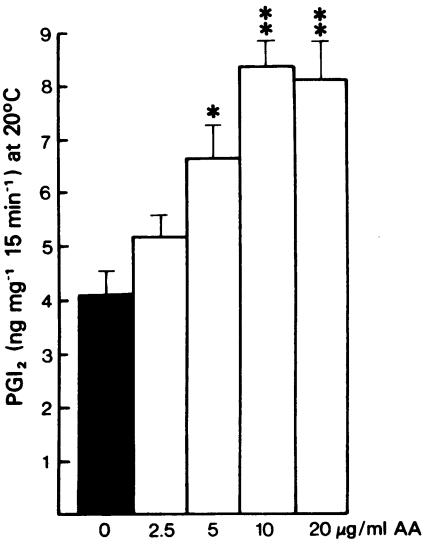

Figure 5 Histogram showing the increased prostacyclin generation which occurred when samples of rat pregnant myometrium (day 22 of pregnancy) were incubated with different doses of arachidonic acid (AA). Mean basal release of prostacyclin (5 experiments) (solid column) is stimulated in the presence of increasing doses of AA (open columns). Vertical lines represent s.e. Significant differences between the basal release and treated-groups are shown (*$P < 0.05$; **$P < 0.005$).

($P < 0.05$; $n = 5$), a 71% increase in production. At 10 µg/ml, production had increased to 8.35 ± 0.50 ng/mg ($P < 0.005$, $n = 5$), a 132% increase over basal synthesis. This represents a 10.7% conversion of exogenous arachidonic acid. Further increases in arachidonic acid concentration did not cause further stimulation of prostacyclin output.

If myometrial tissue from 20 day pregnant rats was used, then a much smaller stimulation of prostacyclin was noted. Synthesis by control samples was 2.07 ± 0.15 ng/mg ($n = 5$) and in the presence of arachidonic acid 10 µg/ml this rose to 3.59 ± 0.37 ng/mg, again a significant increase ($P < 0.05$). This represents a 3.6% of conversion of arachidonic acid by the 20 day pregnant myometrium. Thus, the 22 day pregnant myometrium was approximately 3 times more active than the 20 day pregnant tissue in converting exogenous arachidonic acid into prostacyclin.

Effects of inhibitors

Mepacrine The effects of pre-incubating samples of 20 day pregnant myometrial tissue with varying concentrations of mepacrine are shown in Figure 6. A significant inhibition of production of anti-aggregatory activity was seen at a dose of 320 µg/ml ($P < 0.05$; $n = 6$). Increasing the dose of mepacrine led to further graded inhibitions of prostacyclin output.

Indomethacin and eicosatetraynoic acid (ETA) Indomethacin was tested for its ability to inhibit prostacyclin generation. The results from a typical experiment are illustrated in Figure 7. After obtaining an aggregation response to ADP, the effects of indomethacin were first investigated and a dose of 150 ng in a volume of 5 µl (30 µg/ml) was added 1 min before ADP and as can be seen it did not affect the ADP-induced aggregation. However, as a precaution indomethacin was added together with ADP except when pre-incubated with the myometrial tissue. As the tracing shows, a control sample of myometrium generated 5.8 ng of prostacyclin in the volume tested. However, when the myometrium was pre-incubated with indomethacin 30 µg/ml for 10 min at 37°C before chopping, no detectable anti-aggregatory activity was produced. In 5 experiments with indomethacin, prosta-

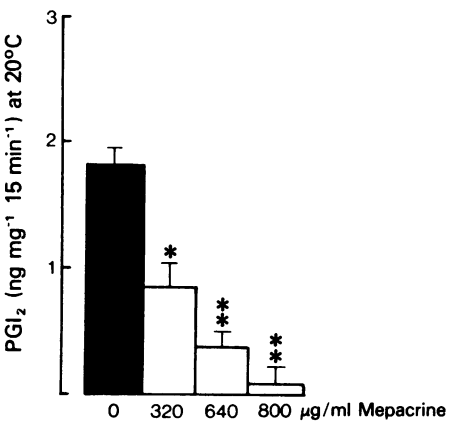

Figure 6 The inhibitory effect of mepacrine on myometrial prostacyclin release. Basal release of prostacyclin (solid column) from the day 20 myometrium is inhibited on incubation with increasing concentrations of mepacrine (open column). Each column represents mean; vertical lines represent s.e. Statistically significant reductions are indicated (*$P < 0.05$; **$P < 0.02$).

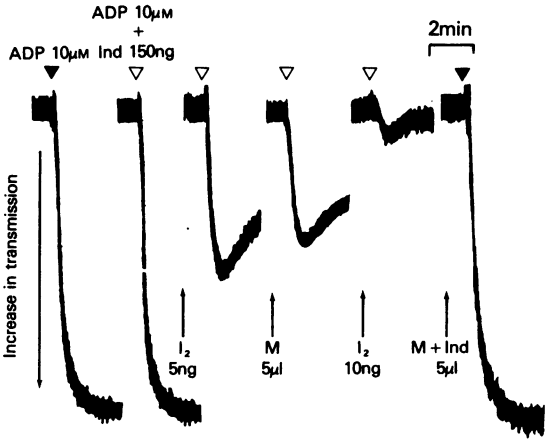

Figure 7 The effect of indomethacin upon myometrial prostacyclin output. The change in light transmission caused by the addition of ADP 10 μM (▼) to rabbit citrated platelet rich plasma was recorded. Indomethacin 150 ng in 5 μl (corresponding to 30 μg/ml) was added together with ADP (▽) except when myometrial tissue was preincubated with indomethacin at 30 μg/ml (M + Ind). Indomethacin itself did not influence ADP-induced aggregation but completely inhibited generation of prostacyclin output when preincubated with the myometrium for 10 min at 37°C.

cyclin production was reduced by 76 ± 7%. Similar experiments with ETA at 30 μg/ml inhibited myometrial prostacyclin production by 65 ± 6% ($n = 5$).

15-Hydroperoxy arachidonic acid (15-HPAA) and tranylcypromine The reduction in output of myometrial anti-aggregatory activity caused by 15-hydroperoxy arachidonic acid (15-HPAA) is shown in Figure 8. As shown, 15-HPAA at a dose of 150 ng potentiated ADP-induced aggregation and was added routinely to PRP samples except when 15-HPAA was pre-incubated with the myometrium. A 5 μl aliquot of control myometrial incubation medium was found to contain 6.4 ng of prostacyclin. However, on pre-incubating a sample of myometrial tissue with 15-HPAA 30 μg/ml, the output of prostacyclin was reduced to 1.25 ng in the same volume. This represents an 80% decrease. In 5 experiments the mean inhibition of myometrial prostacyclin synthesis was 65 ± 6%.

Similar experiments were carried out with tranylcypromine. At a concentration of 320 μg/ml the mean inhibition of myometrial prostacyclin synthesis was 68 ± 9%.

Discussion

The results of these studies add considerably to the characterization of the anti-aggregatory material which we have previously shown to be released from the rat pregnant myometrium (Williams *et al.*, 1978; Williams & El Tahir, 1980a). The experiments also illustrate the marked effect that alteration of the incubation conditions have upon the production of the anti-aggregatory material. When incubations were performed at 37°C, peak concentrations of anti-aggregatory material were detected after 3 min. However, at 20°C peak production was attained after 15 min and was significaatly higher than at 37°C. At either temperature the production of activity was similar at pH 7.4 or pH 8 which is in accordance with other studies (Salmon, Smith, Flower, Moncada & Vane, 1978; Wallach, 1978). However, the pH of the incubation medium was critical in determining the maintenance of the peak concentrations of activity. At pH 8 this covered a 20 min period but at pH 7.4, all detectable activity had disappeared within this time. This indicates the substance closely resembles prostacyclin which is known to be more stable at alkaline pH (Johnson *et al.*, 1976; Moncada *et al.*, 1976). GC-MS analysis provided conclusive proof that the activity generated by the myometrium was prostacyclin, as evidenced by detection of the hydrolysis product, 6-oxo-$PGF_{1\alpha}$ (Johnson *et al.*, 1976). Moreover, quantitative comparison with prostacyclin values estimated by inhibition of platelet aggregation (when incubations were carried out at pH 8 and 20°C) showed close agreement. However, at 37°C estimates

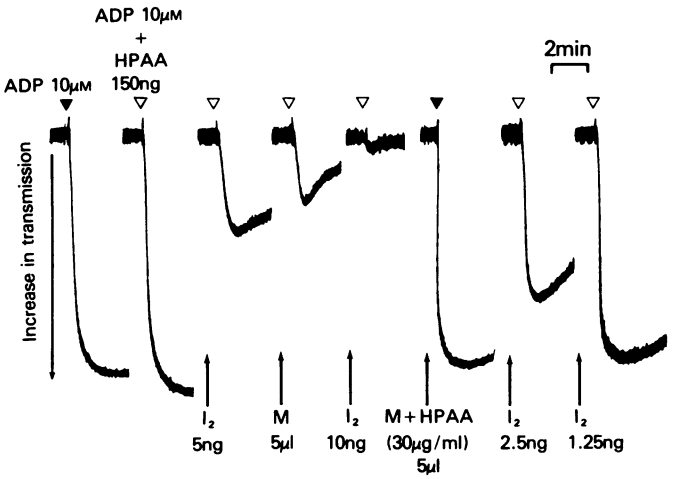

Figure 8 15-Hydroperoxyarachidonic acid (HPAA) inhibits prostacyclin generation by the rat pregnant myometrium. The increase in light transmission caused by the addition of ADP 10 μM to rabbit citrated platelet-rich plasma was recorded (▼). HPAA (150 ng in 5 μl, corresponding to 30 μg/ml) was added concomitantly with ADP (▽) and slightly potentiated the ADP-induced aggregation. Consequently HPAA was added with ADP except when the myometrium was preincubated with this hydroperoxy acid. Preincubation of myometrial tissue with HPAA 30 μg/ml for 10 min at 37°C caused 80% decrease in output compared with the control sample.

by the latter assay were significantly lower but the concentration of 6-oxo-$PGF_{1\alpha}$ detected by GC-MS were similar at 37°C and 20°C. This indicates that although higher temperatures do not affect maximum prostacyclin production, rate of formation is much faster as is the rate of hydrolysis to 6-oxo-$PGF_{1\alpha}$. The similar quantitative values obtained by these methods indicate that the assay of prostacyclin by inhibiton of platelet aggregation is not only accurate, as shown by the low intra- and inter-assay coefficients of variation (Williams & El Tahir, 1980b) but also highly selective. Selectivity was demonstrated by showing that other prostaglandins known to be released from the rat pregnant uterus i.e. PGE_2, $PGF_{2\alpha}$ and PGD_2 (Vane & Williams, 1973; Katori, Harada, Yamashita, Ishibashi & Niazaki, 1978) only exerted anti-aggregatory effects at doses at least 800 times larger than prostacyclin. The wide difference in the potencies of prostacyclin and PGD_2 is due to the insensitivity of rabbit platelets to PGD_2 (Whittle, Moncada & Vane, 1978); human platelets show a much smaller differential sensitivity (Whittle *et al.*, 1978). The possibility of these prostaglandins contributing to the anti-aggregatory response observed was further reduced as they are produced predominantly by the decidual tissue in the uterus (Harney, Sneddon & Williams, 1974; Williams & Downing, 1977) and this tissue was scraped from the myometrium before the incubation commenced. Furthermore, these prostaglandins lose their anti-aggregatory activity after alkalinization, PGE_2 being converted to PGB_2 and PGD_2 to a more polar substance (Pace-Asciak, 1976). The alkaline stability of the myometrial anti-aggregatory material was identical to that of authentic prostacyclin.

Addition of arachidonic acid, the prostaglandin precursor (Bergström, Danielsson & Samuelsson, 1964; van Dorp, Beerthuis, Nugteren & Vonkeman, 1964; Anggard & Samuelsson, 1965) or phospholipase A_2 which cleaves this fatty acid from membrane phospholipids (Flower & Blackwell, 1976) both stimulated myometrial prostacyclin formation in a dose-dependent manner. It was interesting to note that the day of pregnancy on which the uteri were taken influenced the degree of stimulation which these agents produced. In day 20 preparations, phospholipase A_2 caused a larger increase in prostacyclin synthesis than in day 22 samples. This indicates that prostacyclin formation on day 20 of pregnancy is limited by the availability of endogenous phospholipase A_2 and not by reserves of precursor. However, on day 22 phospholipase A_2 produced a smaller increase although basal release of prostacyclin from the myometrium is higher at this time (Williams & El Tahir, 1980a) suggesting that endogenous phospholipase A_2 activity had increased over day 20. This is an important point which must be remembered when studying the actions of drugs which influence myometrial prostacyclin production via phospholipase A_2 stimulation (Williams & El Tahir, 1980b). Conversely, arachidonic acid-induced stimulation was higher on day 22 of pregnancy than on day 20 indicating that although endogenous precursor availability may have increased over

these 2 days (via increased phospholipase A_2 activity) there has also been an increase in myometrial cyclo-oxygenase and/or prostacyclin synthetase activity. Because of this stimulation the synthetic capacity of the myometrium is far greater than can be realised from endogenous precursor conversion. It is probable that the changes in both phospholipase A_2 and prostaglandin and/or prostacyclin synthetase activities are associated with the sharp increase in oestrogen concentrations in the blood which takes place in the later stages of pregnancy in the rat (Fuchs, 1978), as uterine PG synthetase activity increases under the influence of oestrogens (Ham, Cirillo, Zanetti, & Kuehl, 1975).

Preincubation of the myometrial tissue with mepacrine, an inhibitor of phospholipase A_2 (Vargaftig & Dao Hai, 1972; Flower & Blackwell, 1976; Schoene, 1978), reduced myometrial prostacyclin formation in a dose-dependent manner. Although at high dose levels the selectivity of action of mepacrine is lost as it also inhibits the cyclooxygenase enzyme (Flower & Blackwell, 1976), in our experiments the action appeared to be selective as mepacrine did not reduce arachidonic acid-stimulated prostacyclin production (unpublished data). Pretreatment of the myometrial tissue with indomethacin or ETA also inhibited myometrial prostacyclin synthesis, presumably through the inhibitory effects of these drugs on the cyclo-oxygenase enzyme (Ahern & Downing, 1970; Vane, 1971). Tranylcypromine and 15-hydroperoxy arachidonic acid, drugs known to inhibit selectively prostacyclin synthetase (Gryglewski, Bunting, Moncada, Flower, & Vane, 1976; Moncada, Gryglewski, Bunting & Vane, 1976b; Salmon *et al.*, 1978), effectively inhibited myometrial prostacyclin formation. The doses of drug used in our experiments are considerably higher than those found by Moncada *et al.*, (1976) to inhibit prostacyclin synthesis by various microsomal preparations. The higher dose may be due to differences in the susceptibility of the prostacyclin synthetases in these different tissues as reported for the cyclooxygenase enzymes (Flower & Vane, 1974). Alternatively, in whole cell preparations there could be peroxidase enzymes which rapidly reduce the hydroperoxide to the corresponding hydroxy acid (Christopherson, 1968) which is at least 10 times less potent as an inhibitor of prostacyclin synthetase (Gryglewski *et al.*, 1976). Although the selectivity of action of tranylcypromine has recently been questioned (Rajtar & De Gaetano, 1979), the dose used in our experiments was lower than that used in many other experiments to inhibit prostacyclin synthesis (Tomasi, Meringolo, Bartolini & Orlandi, 1978; Weksler, Ley & Jaffe, 1978; Okuma, Yamori, Ohta & Uchino, 1979).

In all these studies to obtain an adequate inhibition pre-incubation with the relevant drug had to be carried out. If the inhibitory drug was present only during the 15 min incubation period at 20°C, no inhibition of prostacyclin release was seen. Thus pre-incubation with the inhibitor is a mandatory step for significant enzyme inhibiton (Smith & Lands, 1972; Blackwell, Flower & Vane, 1975). Reasonably short pre-incubation periods could be used if a temperature of 37°C was employed rather than 20°C as for incubation of the chopped tissue, presumably because diffusion of the drug into the myometrial tissue is considerably slowed at this lower temperature.

In recent studies we have speculated on the physiological importance of uterine prostacyclin synthesis (Williams *et al.*, 1978; Williams & El Tahir, 1980a). Prostacyclin has a weaker oxytocic action than either PGE_2 or $PGF_{2\alpha}$ on the rat pregnant uterus but at subthreshold doses it potentiates the stimulant action of oxytocic drugs (Williams, El Tahir & Marcinkiewicz, 1979). Thus myometrial prostacyclin production may modulate uterine sensitivity during pregnancy. Prostacyclin produced within the uterus may also dilate the uterine blood vessels thus ensuring an adequate blood supply to the developing foetus.

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