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PROSTAGLANDINS, THROMBOXANES AND THE PREGNANT RAT UTERUS AT TERM

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1 Prostaglandin and thromboxane release from the term pregnant (Day 22) rat uterus *in vitro* has been measured by radioimmunoassay and gas chromatography combined with mass spectrometry.

2 Prostacyclin (prostaglandin I_2, PGI_2) and thromboxane A_2 (TXA_2) (measured as their metabolites, 6-oxo-$PGF_{1\alpha}$ and TXB_2, respectively) were released in large amounts, while PGE_2 and $PGF_{2\alpha}$ were released in smaller amounts. PGD_2 was released in the largest quantities.

3 Treatment of the term pregnant rat uterus *in vitro* with the PGI_2 synthesis inhibitors, 15-hydroperoxy arachidonic acid (15-OOH AA) and tranylcypromine caused spasm of the tissue.

4 15-OOH AA caused dose-dependent increases in prostaglandin release, while tranylcypromine caused a fall in the release of PGE_2 but did not affect the release of other prostaglandins. A possible reason for the effect of 15-OOH AA on prostaglandin release is discussed.

5 Indomethacin prevented spontaneous activity of the term pregnant rat uterus *in vitro*. Contractions were restored by prostaglandins and their order of potency was $PGE_2 > PGF_{2\alpha} > PGI_2 > PGD_2 >> 6\text{-oxo-}PGF_{1\alpha} \equiv TXB_2$.

Introduction

The uterus from the term (Day 22) pregnant rat, freed of conceptuses, exhibits spontaneous activity *in vitro*. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and PGE_2 are released into the bathing fluid and the amounts of $PGF_{2\alpha}$ released have been measured (Vane & Williams, 1973). The spontaneous contractions of the uterus and prostglandin release are inhibited by the addition of a cyclo-oxygenase inhibitor, such as indomethacin and meclofenamate, to the organ bath. Contractions can be restored by the further addition of PGE_2 or $PGF_{2\alpha}$, with PGE_2 being the more potent. Since the output of PGE_2 and $PGF_{2\alpha}$ and the spontaneous contractions of the uterus in this *in vitro* system are greater on Day 22 than on earlier days of pregnancy, a role for these prostaglandins in parturition has been suggested (Williams, Sneddon & Harney, 1974). However, these studies were carried out before thromboxanes and prostacyclin (PGI_2) were discovered. The following experiments have therefore been performed to measure the release of PGI_2 and thromboxane A_2 (TXA_2) as well as PGD_2, $PGF_{2\alpha}$ and PGE_2, from the pregnant rat uterus at term *in vitro*. The effects of the oxy-cyclase inhibitors, 15-hydroperoxy arachidonic acid (15-OOH AA) and tranylcypromine (Moncada, Gryglewski, Bunting & Vane, 1976) on contractions and prostaglandin release from the Day 22 pregnant rat uterus, *in vitro*, have also been examined.

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Methods

Mature female, Wistar rats exhibiting regular 4 day oestrous cycles were housed with light changes occurring at 08 h 00 min and 20 h 00 min. Vaginal smears were taken daily and examined microscopically. Day 1 of the oestrous cycle was the day of maximum cornification, preceding the day of leucocytic infiltration into the vagina. The rats were mated singly by placing them with a male rat of proven fertility on the afternoon of Day 4. Mating was confirmed to have taken place by the appearance of spermatozoa in the vaginal smear taken on the following day. This day now became Day 1 of pregnancy.

Initially, 3 rats were allowed to go to term and delivered during the dark period between Days 22 and 23. Rats for experimental purposes were used on the morning of Day 22. Each rat was killed and the uterus was removed and placed in Krebs solution of the following composition (g/l): NaCl 6.9, KCl 0.354, $MgSO_4\ 7H_2O$ 0.294, KH_2PO_4 0.162, $CaCl_2$ 0.282, $NaHCO_3$ 2.1, glucose 2.0. The two uterine horns were divided, cut longitudinally and freed of foetuses and placentae. Each horn was blotted dry, weighed and suspended in an 80 ml organ bath containing Krebs solution at 37°C, aerated with 95% O_2 and 5% CO_2. A tension of 2 g was applied to each horn and isotonic contractions were recorded on a Vitatron pen recorder. The uterine horns were then used in one of the following experiments:-

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Prostaglandin and thromboxane release from the Day 22 pregnant rat uterus

Contractions of the uterine tissue were recorded for 4 consecutive periods of 15 min. After each period, the bath fluid was removed and retained, the organ bath was refilled and the four collected samples were pooled. Prostaglandins and TXB_2 were extracted as described by Poyser & Scott (1980), and stored at −20°C before being assayed. The extraction procedure gives greater than 90% recoveries of $PGF_{2\alpha}$, PGE_2, PGD_2 and TXB_2, while the recovery of 6-oxo-$PGF_{1\alpha}$ is 65 to 75% (Poyser & Scott, 1980). Bath fluid surrounding 12 uterine horns obtained from 12 rats was treated in this way.

For a further 3 uterine horns, the bath fluid collected over 4 consecutive 15 min periods was not pooled, and the prostaglandin and TXB_2 content of each '15 min' sample was determined after extraction in order to ascertain the rate of their release.

Bath fluid was collected from another uterine horn for three consecutive 15 min periods. At the beginning of the second and third periods, 80 ml Krebs solution containing indomethacin in concentrations of 0.6 μg/ml and 1.2 μg/ml respectively were added to the organ bath. Following extraction, the amounts of PGE_2 and $PGF_{2\alpha}$ released from the uterine tissue were measured to verify that indomethacin inhibits prostaglandin release in this experimental situation.

Measurement of prostaglandins and thromboxane B_2

$PGF_{2\alpha}$, PGE_2 and 6-oxo-$PGF_{1\alpha}$ were measured by radioimmunoassay (RIA) (Dighe, Emslie, Henderson, Rutherford & Simon, 1975; Mitchell, Poyser & Wilson, 1977; Dighe, Jones & Poyser, 1978; Poyser & Scott, 1980). Although the $PGF_{2\alpha}$ antibody does not distinguish between $PGF_{2\alpha}$ and $PGF_{1\alpha}$, it is probable that only $PGF_{2\alpha}$ was being measured as the rat uterus synthesizes very little $PGF_{1\alpha}$ (Fenwick, Jones, Naylor, Poyser & Wilson, 1977). Cross-reactivity of the prostaglandin antisera with other prostaglandins and their metabolites is low (Poyser & Scott, 1980).

The inter-assay coefficients of variation for the $PGF_{2\alpha}$, PGE_2 and 6-oxo-$PGF_{1\alpha}$ radioimmunoassays were 8.4, 4.2 and 6.1%, respectively. In each assay, biological samples were assayed in duplicate. If the coefficient of variation between the values obtained in each pair of results exceeded 15%, then the biological sample was reassayed. However, for the majority of samples, this intra-assay coefficient of variation between paired values was less than 10%.

PGD_2 and TXB_2 were measured by gas chromatography-mass spectrometry (g.c. m.s.) on a VG Micromass 7070F dual focusing machine, using the multiple ion detector unit. The column was packed with 3% OV1 on Supelcoport (Supelco Inc., Belleforte, U.S.A.), the helium gas flow was 30 ml/min and the column temperature was 260°C. The methyl ester-butyloxime-trimethylsilyl ether (Me-BuO-TMS) derivatives and ethyl ester-butyloxime-trimethylsilyl (Et-BuO-TMS) derivatives of PGD_2 and TXB_2 were prepared as described previously (Fenwick *et al.*, 1977). Standard quantities of 0, 25, 50, 100 and 200 ng PGD_2 (as the Me-BuO-TMS derivatives, carbon value 25.7) together with an internal standard of 100 ng PGD_2 (as the Et-BuO-TMS derivative, carbon value 26. 1) were injected into the apparatus, and the corresponding m/e ions at 420 and 434 were recorded. A standard curve was obtained by plotting the ratio of the heights of the 420 and 434 ions against the standard quantities of PGD_2. A standard curve for TXB_2 was obtained in a similar manner by recording the m/e ion at 301 produced by both the Me-BuO-TMS derivative (carbon value 26.4) and Et-BuO-TMS derivative (carbon value 26.9).

Biological samples were assayed by adding 300 ng each of the Et-BuO-TMS derivative of PGD_2 and TXB_2 to two-fifths of the sample (previously converted to the Me-BuO-TMS derivative), by injecting one-third of the sample into the apparatus and by monitoring the m/e ions at 301, 420 and 434. By calculating the ratio of the peak heights of the appropriate ions at the correct carbon values for the authentic compound, and by reference to the standard curves, the amounts of PGD_2 and TXB_2 in each biological sample were obtained.

Determination of the potency of prostaglandins and thromboxane B_2 in restoring uterine contractions

Indomethacin (final concentration 5 or 10 μg/ml) was added to the organ bath containing a Day 22 uterine horn to render the horn quiescent. Prostaglandins or TXB_2 were dissolved in 0.9% saline (adjusted to pH 7.5 with $NaHCO_3$) and added to the organ bath. The minimum concentration of prostaglandin or TXB_2 necessary to restore contractions of the uterine tissue was found and tested at least three times on three different preparations. The relative potencies of $PGF_{2\alpha}$, PGE_2, PGI_2, PGD_2, 6-oxo-$PGF_{1\alpha}$ and TXB_2 in restoring contractions of the indomethacin-treated, Day 22 pregnant rat uterus, *in vitro*, were determined.

Effect of 15-OOH AA and tranylcypromine on uterine contractions and prostaglandin release

Preparation of 15-OOH AA 15-OOH AA was synthesized by reacting 10 mg arachidonic acid (99% pure, Sigma, Poole, Dorset) with 0.25 mg soya bean lipoxidase (Sigma, Poole, Dorset) in 150 ml 0.1 M trihydroxymethylamine phosphate (Tris) buffer at

pH 8.0 at room temperature with continuous stirring. The reaction was monitored by u.v. light absorption at 234 nm. When the reaction was completed (approximately 15 min), the pH of the reaction mixture was lowered to 4.0 with 0.1 M HCl and the products extracted by shaking twice with an equal volume of diethyl ether. The ether extracts were pooled, washed with 0.2 volume of water and dried over anhydrous sodium sulphate. The ether was evaporated off at room temperature on a rotary evaporator, and the residue dissolved in 1.0 ml hexane for further purification by high-performance liquid chromatography (h.p.l.c.). The column was packed with Partisil PAC (Whatman Labsales, Ltd., Maidstone, Kent) and the h.p.l.c. was performed on an 848 Pump Model from Du Pont Instruments. The solvent system was hexane and isopropanol (10:1), containing 0.1% glacial acetic acid. The column flow rate was 2 ml/min. A maximum of 50 μl of the extract was injected on to the column each time, necessitating repeated injections. The products were detected by u.v. absorption at 234 nM. The first peak to appear was that of 15-hydroxy arachidonic (15-OH AA) after 3.5 min, followed by 15-OOH AA 2 min later. The second peak from each injection was collected, pooled and the amount of 15-OOH AA present quantified by measuring the u.v. absorption of 234 nm (Molar extinction coefficient of 15-OOH AA is 25,000). 15-OOH AA was stored in methanol at -20°C before use and, under these conditions, remained stable for at least 2 weeks.

To verify that the 15-OOH AA was authentic, a known amount was reduced by shaking with a saturated solution of stannous chloride for 5 to 10 min to form 15-OH AA. The identity of 15-OH AA was confirmed by g.c.m.s. after forming the Me-TMS derivative (as described for prostaglandins by Fenwick *et al.*, 1977), thus establishing the identity of the non-reduced material as 15-OOH AA.

Uterine studies The bath fluid surrounding a uterine horn was collected for a 15 min control period. 15-OOH AA (25, 30 or 40 μg/ml dissolved in 0.2 to 0.6 ml polyethylene glycol) or tranylcypromine (500 μg/ml) was then added to the organ bath and the uterine contractions recorded for a further 15 min period. At the end of this period, the bath fluid was collected, solvent extracted and the prostaglandin content measured as before, except for the insertion of a further purification step as described below. The effect of the two compounds at each dose level was investigated 3 times. The addition of polyethylene glycol or saline alone did not affect the uterine tissue. The effect of 15-OOH AA and tranylcypromine on the contractility of the uterus treated with indomethacin (10 μg/ml) was also examined, each on a further three preparations.

Removal of 15-OOH AA from extracted samples Samples of extracted bath fluid were assayed by RIA for $PGE_{2\alpha}$, PGE_2 and 6-oxo-$PGF_{1\alpha}$. 15-OOH AA cross-reacted 0.09% and 0.005% with the $PGF_{2\alpha}$ and PGE_2 antibodies, respectively. However, although these values are low, 15-OOH AA at the concentrations used may still have interfered with the assays. 15-OOH AA was therefore separated from prostaglandins and TXB_2 by silicic acid column chromatography. 15-OOH AA was recovered in Fraction 1 (150 ml 80% toluene and 20% ethyl acetate) and the prostaglandins and TXB_2 in Fraction 2 (200 ml 50% toluene, 48% ethyl acetate and 2% methanol). By assaying the control samples of extracted fluid before and after silicic column chromatography (i.e. no 15-OOH AA present at all) it was found that the average recoveries (mean $\pm$ range, $n = 12$) from the columns were $PGF_{2\alpha}$ 30 $\pm$ 4%, PGE_2 85 $\pm$ 12%, 6-oxo-$PGF_{1\alpha}$ 30 $\pm$ 6%, PGD_2 80 $\pm$ 2% and TXB_2 36 $\pm$ 5%. The recovery of TXB_2 was too low for results to be obtained for every sample in the g.c.m.s. assay, so only the prostaglandin results have been recorded. All control, 15-OOH AA and tranylcypromine treated samples were subjected to silicic acid column chromatography before being assayed for $PGF_{2\alpha}$, PGE_2 and 6-oxo-$PGF_{1\alpha}$ by RIA and for PGD_2 by g.c.m.s. Results have not been corrected for recovery as a comparison is being made between control and treated samples.

Statistical tests

Student's t test was used to test for significant differences between groups of results.

Results

Prostaglandin and thromboxane release from the Day 22 pregnant rat uterus

The amounts (mean $\pm$ s.e. mean, $n = 12$) of prostaglandins and TXB_2 released from the Day 22 pregnant rat uterus *in vitro* are shown in Figure 1. PGD_2 was released in the greatest quantity, followed in descending order by TXB_2, 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2. The release of 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$ and PGE_2 from the uterus over four consecutive 15 min periods tended to decrease with time (Table 1). Indomethacin inhibited the release of PGE_2 and $PGF_{2\alpha}$ by 67% and 78% respectively at a concentration of 0.6 μg/ml, and by 90% and 96% respectively at a concentration of 1.2 μg/ml.

Determination of the potency of prostaglandins and thromboxane B_2 in restoring uterine contractions

Spontaneous contractions of the uterus were inhi-

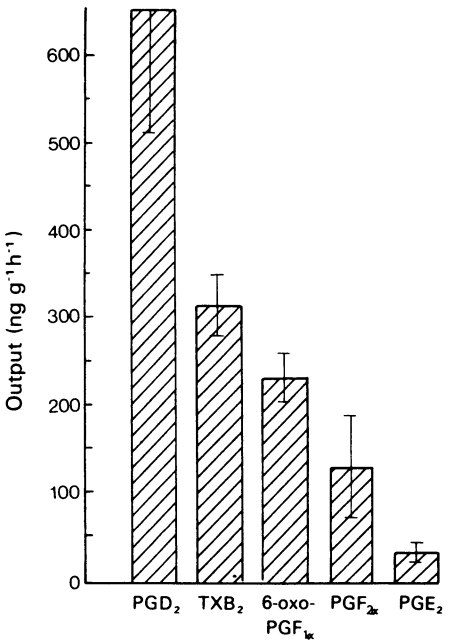

Figure 1 Prostaglandin and thromboxane output (mean of, n = 12) from the spontaneously contracting Day 22 pregnant rat uterus *in vitro*; vertical lines show s.e. mean.

bited by indomethacin at 5 or 10 μg/ml. Contractions of all preparations could be restored by the addition of prostaglandins or TXB_2 to the organ bath (Figure 2). PGE_2 was the most potent, restoring contractions at 2 ng/ml, whereas $PGF_{2\alpha}$ was effective at 8 ng/ml. The addition of 8 ng/ml PGI_2 to the organ bath followed by a further addition of 16 ng/ml elicited contractions from the uterus. Contractions were not obtained by adding 8 to 16 ng/ml as single doses. PGD_2 contracted the uterus at a dose level of 60 ng/ml, while 6-oxo-$PGF_{1\alpha}$ and TXB_2 were active at 500 ng/ml. Contractions obtained with PGI_2 and PGD_2 were qualitatively different from those obtained with $PGF_{2\alpha}$ and PGE_2 since they were more regular and there was little change in basal tone of the tissue.

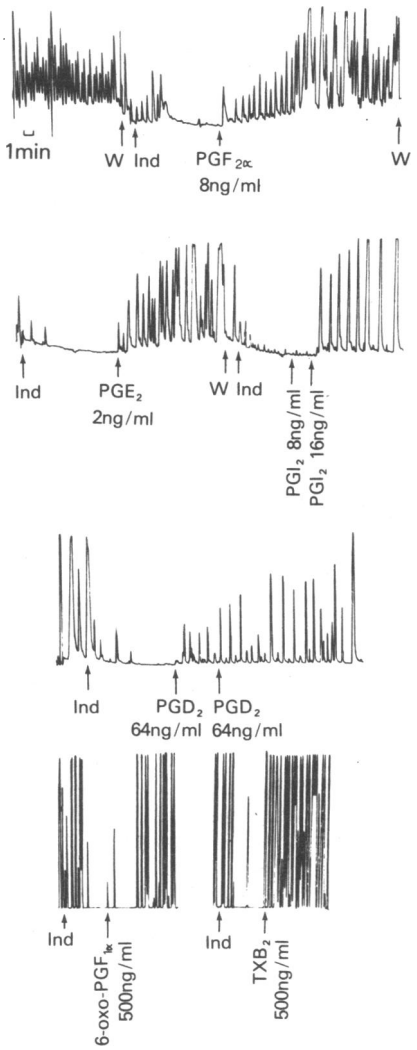

Figure 2 Minimum doses prostaglandin and thromboxane required to restore uterine contractions to the Day 22 pregnant rat uterus treated with indomethacin (Ind 5 or 10 μg/ml) *in vitro*. (At W, the tissue was washed).

Table 1 Mean (±s.e. mean, n = 3) prostaglandin release (ng g^{-1} min^{-1}) from the Day 22 pregnant rat uterus over four consecutive 15 min periods

Prostaglandin	*Amounts released during periods:-*			
	1	*2*	*3*	*4*
6-oxo-$PGF_{1\alpha}$	3.5 ± 0.6	3.1 ± 0.3	2.1 ± 0.5	2.0 ± 0.2
$PGF_{2\alpha}$	3.1 ± 0.13	2.7 ± 0.2	1.9 ± 0.1	1.9 ± 0.2
PGE_2	1.2 ± 0.2	1.0 ± 0.1	1.2 ± 0.4	0.9 ± 0.2

Effect of 15-OOH AA and tranylcypromine on uterine contractions and prostaglandin release

15-OOH AA, at all three concentrations, and tranylcypromine caused a large increase in uterine tone in the presence and absence of indomethacin. 15-OOH AA significantly increased the release of all prostaglandins, while tranylcypromine significantly decreased the release of PGE_2. Neither compound inhibited the release of PGI_2, as indicated by measuring 6-oxo-$PGF_{1\alpha}$ (Figure 3).

Discussion

This study has shown that prostaglandins and thromboxanes are released from the Day 22 pregnant rat uterus *in vitro*. The amounts of $PGF_{2\alpha}$ released as measured by RIA were similar to but slightly lower than those previously reported by Vane & Williams (1973) who used bioassay. PGE_2 was released in smaller amounts than $PGF_{2\alpha}$ while PGI_2 and TXA_2 (measured as their metabolites, 6-oxo-$PGF_{1\alpha}$ and TXB_2, respectively) were released in larger amounts. The most abundant prostaglandin released was PGD_2. Its release from the pregnant rat uterus has

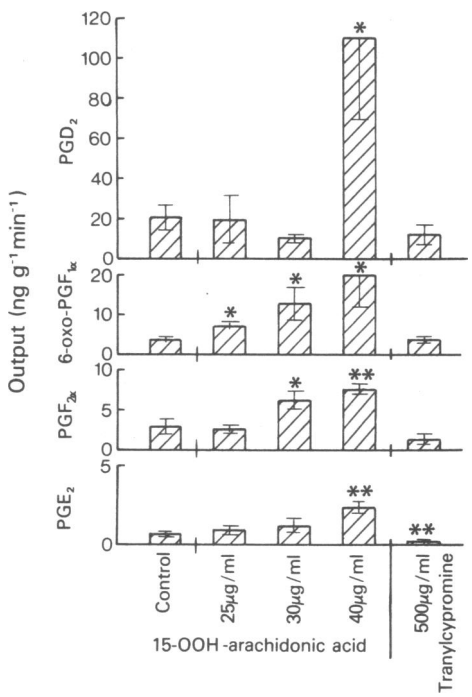

Figure 3 Effect of 15-hydroperoxy arachidonic acid (15-OOH-arachidonic acid) and tranylcypromine on prostaglandin output from the day 22 pregnant rat uterus *in vitro*. Significant changes from control values are indicated: $*P<0.05$; $**P<0.01$ ($n = 3$).

previously been reported but was not quantified (Katori, Harada, Yamashita, Ishibashi & Miyazaki, 1978). Rat decidual microsomes on Day 22 of pregnancy, synthesized $PGF_{2\alpha}$, PGE_2, PGD_2, 6-oxo-$PGF_{1\alpha}$ and TXB_2, *in vitro*, and the identity of these compounds, with the exception of PGD_2, was confirmed by g.c.m.s. (Williams & Downing, 1977). The myometrium synthesized mainly PGI_2 (Williams, Dembinska-Kieć, Zmuda & Gryglewski, 1978).

TXB_2 is not detectable in rat decidual or placental tissue on Day 15 of pregnancy but measurable quantities are present by Day 20 and these levels increase up to the time of parturition (Zamecnik & Kennedy, 1980). 6-oxo-$PGF_{1\alpha}$ levels in the placenta increase similarly, whereas 6-oxo-$PGF_{1\alpha}$ levels in the decidua remain constant over the last stages of pregnancy. Inhibition of platelet aggregation has been used to measure and compare PGI_2 production by different fractions of the pregnant uterus (Williams *et al.*, 1978), but the method failed to detect PGI_2 production by the rat placenta in contrast to Zamecnik & Kennedy (1980) who detected and measured the breakdown product of PGI_2 (6-oxo-$PGF_{1\alpha}$) in rat placenta by both RIA and g.c.m.s.

The potencies of PGE_2, $PGF_{2\alpha}$ and PGI_2 in restoring uterine contractions to the Day 22, indomethacin-treated uterus were similar to those found by Vane & Williams (1973) and Williams, El-Tahir & Marcinkiewicz (1979), although 6-oxo-$PGF_{1\alpha}$ was more potent in our study than in the study of Williams *et al.* (1979). TXB_2 is similar in potency to 6-oxo-$PGF_{1\alpha}$ while PGD_2 was the weakest of the four primary prostaglandins tested. PGI_2 produced regular contractions of the uterus, in contrast to PGE_2 and $PGF_{2\alpha}$, but whether PGI_2 produced by the myometrium is involved in the synchronous, regular contractions of parturition is not known. PGI_2 sensitizes the rat uterus to the action of other uterine stimulants such as oxytocin (Williams *et al.*, 1979).

15-OOH AA and tranylcypromine caused a large increase in tone of the spontaneously contracting rat uterus. 15-OOH AA also caused a dose-dependent increase in the release of all prostaglandins from the uterus. This result was surprising as 15-OOH AA is supposedly a specific inhibitor of PGI_2 synthesis (Moncada *et al.*, 1976; Gryglewski, Bunting, Moncada & Vane, 1976). It is unlikely that 15-OOH AA is converted into prostglandins as the corresponding compound, 15-hydroperoxy-8, 11, 13-eicosatrienoic acid is not converted to the l-series prostaglandins (Hamberg & Samuelsson, 1967). 15-OOH AA inhibits the lipoxygenase pathway of arachidonic acid metabolism in the lungs in the same concentrations as it inhibits PGI_2 synthesis (Burka & Flower, 1979). Lipoxygenase is a soluble enzyme (Nugteren, 1975) whereas the PGH_2 to PGI_2 oxy-cyclase enzyme is present in the microsomes

(Gryglewski *et al.*, 1976). In the intact tissue, 15-OOH AA may have easier access to the lipoxygenase enzyme, thereby inhibiting this pathway in more arachidonic acid becoming available for conversion into prostaglandins. In addition, hydroperoxides stimulate PGG_2 formation from arachidonic acid (see Lands, 1979), so 15-OOH AA may be stimulating prostaglandin and TXB_2 synthesis directly. Further study is required into these suggestions.

Treatment of the isolated rat uterus with tranylcypromine at a concentration which produces 100% inhibition of PGI_2 synthesis by pig aortic microsomes (Gryglewski *et al.*, 1976) did not reduce PGI_2 release. However, a fall in PGE_2 output was observed. Rajtar & de Gaetano (1979) have previously reported that tranylcypromine is not a selective inhibitor of PGI_2 synthesis in the rat.

In conclusion, the order of prostaglandin and thromboxane release from the isolated, spontaneously contracting rat uterus on Day 22 of pregnancy was $PGD_2 > TXA_2 > PGI_2 > PGF_{2\alpha} > PGE_2$. This was the reverse of their uterine spasmogenic potency, $PGE_2 > PGF_{2\alpha} > PGI_2 > PGD_2$. $PGF_{2\alpha}$ and PGE_2 are released into the uterine venous blood of rats at the end of gestation (Shaikh, Naqvi & Saksena, 1977), but the output of PGI_2, PGD_2 and TXA_2 from pregnant rat uterus *in vivo* requires investigation.

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References

BURKA, J.F. & FLOWER, R.J. (1979). Effects of modulators of arachidonic acid on the synthesis and release of slow-reacting substance of anaphylaxis. *Br. J. Pharmac.*, **65**, 35–41.

DIGHE, K.K., EMSLIE, H.A., HENDERSON, L.K., RUTHERFORD, F. & SIMON, L. (1975). The development of antisera to prostaglandins B_2 and $F_{2\alpha}$ and their analysis using solid-phase and double antibody radioimmunoassay methods. *Br. J. Pharmac.*, **55**, 503—514.

DIGHE, K.K., JONES, R.L. & POYSER, N.L. (1978). Development of a radioimmunoassay for measuring 6-oxo-prostaglandin $F_{1\alpha}$. *Br. J. pharmac.*, **63**, 406P.

FENWICK, L., JONES, R.L., NAYLOR, B., POYSER, N.L. & WILSON, N.H. (1977). Production of prostaglandins by the pseudopregnant rat uterus, *in vitro* and the effect of tamoxifen with the identification of 6-keto-prostaglandin $F_{1\alpha}$ as a major product. *Br. J. Pharmac.*, **59**, 191–199.

GRYGLEWSKI, R., BUNTING, S., MONCADA, S., FLOWER, R.J. & VANE, J.R. (1976). Arterial walls are protected against deposition of platelet thrombi by a substance (PGX) which they make from prostaglandin endoperoxides. *Prostaglandins*, **12**, 685–713.

HAMBERG, M. & SAMUELSSON, B. (1967). On the mechanism of the biosynthesis of prostaglandins E_1 and $F_{1\alpha}$. *J. biol. Chem.*, **242**, 5336–5343.

KATORI, M., HARADA, Y., YAMASHITA, Y., ISHIBASHI, M. & MIYAZAKI, H. (1978). Release of prostaglandin D_2 as a major product from the isolated rat pregnant uterus. *Prostaglandins*, **15**, 697.

LANDS, W.E. (1979). The biosynthesis and metabolism of prostaglandins. *A. Rev. physiol.*, **41**, 633–652.

MITCHELL, S., POYSER, N.L. & WILSON, N.H. (1977). Effect of *p*-bromophenacyl bromide, an inhibitor of phospholipase A_2, on arachidonic acid release and prostaglandin synthesis by the guinea-pig uterus *in vitro*. *Br. J. Pharmac.*, **59**, 107–113.

MONCADA, S., GRYGLEWSKI, R., BUNTING, S. & VANE, J.R. (1976). A lipid peroxide inhibits the enzyme in blood vessel microsomes that generates from prostaglandin endoperoxides the substance (PGX) which prevents platelet aggregation. *Prostaglandins*, **12**, 715–737.

NUGTEREN, D.J. (1975). Arachidonate lipoxygenase in blood platelets. *Biochem. biophys. Acta*, **380**, 299–307.

POYSER, N.L. & SCOTT, F. (1980). Prostaglandin and thromboxane production by the rat uterus and ovary *in vitro* during the oestrous cycle. *J. Reprod. Fert.*, **60**, 33–40.

RAJTAR, G. & DE GAETANO, G. (1979). Tranylcypromine is not a selective inhibitor of prostacyclin in rats. *Thrombosis Res.*, **14**, 245–248.

SHAIKH, A.A. NAQVI, RH. & SAKSENA, S.K. (1977). Prostaglandins E and F in uterine venous plasma in relation to peripheral levels of progesterone and 20α-hydroxyprogesterone in the rat throughout pregnancy and parturition. *Prostaglandins*, **13**, 311–320.

VANE, J.R. & WILLIAMS, K.I. (1973). The contributions of prostaglandin production to contractions of the isolated uterus of the rat. *Br. J. Pharmac.*, **48**, 629–639.

WILLIAMS, K.I., SNEDDON, J. & HARNEY, P.J. (1974). Prostaglandin production by the pregnant rat uterus *in vitro* and its relevance to parturition. *Pol. J. Pharmac. Pharm.*, **26**, 207–215.

WILLIAMS, K.I. & DOWNING, I. (1977). Prostaglandin and thromboxane production by rat decidual microsomes. *Prostaglandins*, **14**, 813–817.

WILLIAMS, K.I., DEMBINSKA-KIEĆ, ZMUDA, A. & GRYGLEWSKI, R.J. (1978). Prostacyclin formation by myometrial and decidual fractions of the pregnant rat uterus. *Prostaglandins*, **15**, 343–350.

WILLIAMS, K.I., EL-TAHIR, K.E.H. & MARCINKIEWICZ, E. (1979). Dual actions of prostacylin (PGI_2) on the rat pregnant uterus. *Prostaglandins*, **17**, 667–672.

ZAMECNIK, J. & KENNEDY, T.G. (1980). Concentrations of 6-keto-$PGF_{1\alpha}$ in amnitoic fluid, decidual tissue and placenta in rats in the later stages of pregnancy. In *Advances in Prostaglandin and Thromboxane Research*, Vol. 8. ed. Paoletti, R., Ramwell, P.W. & Samuelsson, B. pp. 1423–1429. New York: Raven Press.

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