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# THE INFLUENCE OF METYRAPONE ON THE SYNTHESIS AND RELEASE OF PROSTAGLANDINS FROM THE PREGNANT RAT UTERUS *in vitro*

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1 Metyrapone (150 mg/kg, s.c. or i.p.) an inhibitor of corticosteroid biosynthesis, significantly reduced the release of prostaglandins of the F-type from isolated preparations of pregnant rat uteri *in vitro*, on day 22 – the expected day of delivery.

2 Metyrapone and indomethacin administered *in vitro* both inhibited the conversion of $^{14}$C-arachidonic acid to prostaglandin $E_2$ by homogenates of day 22 pregnant rat uteri. Metyrapone was approximately 150 times less potent than indomethacin. Although indomethacin also inhibited prostaglandin $F_{2\alpha}$ production, metyrapone stimulated synthesis of this prostaglandin. The differential inhibition of prostaglandin synthesis by metyrapone may reflect sensitivity of the inhibitor to changes in experimental conditions.

3 Inhibition of prostaglandin synthesis may explain the effects of metyrapone on parturition in the rat.

## Introduction

An increasing body of evidence appears to indicate a role of the foetal adrenal glands in the mechanism of the initiation of parturition in the sheep and other long-gestation species (see Liggins, Fairclough, Grieves, Kendall & Knox, 1973). In the rat, however, the role of the foetal hypophyseal-adrenal system is obscure. Foetal plasma and adrenal tissue corticosterone concentrations have been shown to increase towards the end of pregnancy, but fall during the last 2 days (Kalavsky, 1971; Cohen, 1973). Administration of metyrapone, an inhibitor of corticosteroid biosynthesis, causes foetal hypertrophy (Dupouy, 1972) and prolongs pregnancy in the rat (Parvez, Parvez & Roffi, 1972), though removal of the foetal brain did not alter gestation length (Swaab & Honnebier, 1973) and surgical removal of the foetuses is reported not to delay parturition in this species (Selye, Collip & Thomson, 1935; Kirsch, 1938). However, this latter procedure results in a significant reduction in the *in vitro* uterine release of prostaglandin F (Parnham, Sneddon & Williams, 1975). Prostaglandin F is released *in vitro* in increasing amount during the last 5 days of pregnancy in the rat, reaching a maximum on the expected day of delivery and this release is related to an increase in uterine activity *in vitro* (Vane & Williams, 1973; Harney, Sneddon & Williams, 1974; Williams, Sneddon & Harney, 1974). Administration of antibody to prostaglandin $F_{2\alpha}$ delayed parturition in the rat (Dunn, Humphries, Judkins, Kendall & Knight, 1973) and administration of indomethacin (Aiken, 1972; Chester, Dukes, Slater & Walpole, 1972), an inhibitor of prostaglandin biosynthesis (Vane, 1971), both reduced the release of prostaglandin F and delayed parturition in the rat.

The present study was carried out to investigate the effect of metyrapone on the release of prostaglandin F from the pregnant rat uterus *in vitro*. Some of these results have been demonstrated to the British Pharmacological Society (Parnham & Sneddon, 1975).

## Methods

### *Mating*

Porton strain virgin female rats 6-8 weeks old were mated singly overnight with proven males and successful matings were determined by the appearance of vaginal plugs the following morning (designated day 1). Housing conditions have been described previously (Parnham *et al.*, 1975).

### *Drug administration*

For studies on *in vitro* prostaglandin F release pregnant rats were caged singly in two groups with

nesting materials on day 19 of pregnancy. Group I: rats received daily injections of metyrapone (150 mg/kg, s.c. or i.p.). Group II: rats received an equivalent volume of vehicle, 0.33 M (+)-tartaric acid (0.75 ml/kg, s.c. or i.p.). All injections were given between 16.00 h and 17.00 h on days 20 and 21 of pregnancy.

*Isolated uteri*

Three control and 4 metyrapone-treated rats were killed on the morning of day 22 by a blow on the head, the uteri removed, and individual horns mounted in a 75 ml organ bath as described by Vane & Williams (1973). Bath fluid was collected and extracted for prostaglandins as described previously (Vane & Williams, 1973).

*Bioassay of extracts*

Extracts were reconstituted with 0.9% NaCl solution and assayed on the rat isolated colon against authentic prostaglandin $F_{2\alpha}$ as described previously (Harney *et al.*, 1974).

*Homogenization*

Two untreated pregnant rats were killed on the morning of day 22 by a blow on the head, the uteri removed and immediately placed in ice-cold phosphate buffer, pH 7.5, containing (g/l) $Na_2HPO_4$ (14.2), and $NaH_2PO_4.2H_2O$ (15.6). The uteri were opened longitudinally and the foetuses and placentae removed. The tissue was then chopped and homogenized in 7 ml ice-cold phosphate buffer (pH 7.5), using an Ultra-Turrax homogenizer (2 x 10 s separated by 60 seconds).

*Radiometric assay*

This assay is a modification of the method of Blackwell, Flower, Parsons & Vane (1975). The incubation mixture (in 25 ml Ehrlenmeyer flasks) consisted of the following: 50 $\mu$l [$^{14}$C]-arachidonic acid in *n*-hexane (10-14,000 d/min) evaporated to dryness under nitrogen and resuspended, with shaking, in 1 ml phosphate buffer, pH 7.5; 1 $\mu$g prostaglandin $F_{2\alpha}$ (in 0.1 ml) and 1 $\mu$g prostaglandin $E_2$ (in 0.1 ml) to minimize metabolism of labelled product; 0.1 ml phosphate buffer (for boiled blank and control), 0.1 ml metyrapone (final concentrations 0.05 mM, 0.2 mM and 0.5 mM) or 0.1 ml indomethacin (final concentrations 0.001 mM, 0.002 mM and 0.005 mM); 1 ml homogenate (giving a final protein concentration of about 20 mgm). Protein determination was carried out by the method of Lowry, Rosebrough, Farr & Randall (1951). Each incubation flask was then shaken and incubated for 10 min in a shaking water bath at 36° C against a boiled blank. (Time course studies showed that both prostaglandin $E_2$ and $F_{2\alpha}$ synthesis reached 80% of maximum within 15 minutes.) The reaction was stopped by pouring the incubates into centrifuge tubes, washing the flasks with 1 ml phosphate buffer and boiling on a water bath for 60 seconds. A further 1 ml buffer was added to each tube to cool down the contents, followed by mixing and centrifugation at 1000 *g* for 5 minutes. The supernatant was decanted, acidified to pH 3 with 1 N HCl, and 1 ml saturated NaCl solution was added to facilitate protein precipitation. This mixture was once again centrifuged at 1000 *g* for 5 minutes. The supernatant was then decanted and extracted twice with two volumes ethyl acetate. The two organic fractions were pooled, evaporated to dryness and resuspended in 1 ml 2% methanol in chloroform. (Alternatively the samples were stored at −21° C before being resuspended.) Each sample thus resuspended was subjected to column chromatography on 8 cm silicic acid (325 mesh) columns using 2%, 4% and 10% methanol in chloroform in 10 ml volumes as successive eluants (K.I. Williams, personal communication). Prostaglandin $E_2$ was collected in the 4% fraction and prostaglandin $F_{2\alpha}$ in the 10% fraction. Each fraction was evaporated to dryness, resuspended in scintillation fluid and counted on a Packard Tri-Carb liquid scintillation counter. Two experiments were carried out to check the purity of the column fractions by subjecting the three fractions obtained from control incubates and boiled blank to thin layer chromatography on 0.25 mm silicic acid plates using system E of Nugteren & Hazelhof (1973) against authentic prostaglandin $E_2$, $D_2$ and $F_{2\alpha}$. The plates were divided into 1 cm zones which were scraped into scintillation vials for counting.

**Materials**

Metyrapone was obtained from CIBA, Horsham; indomethacin from Merck, Sharpe & Dohme; [$^{14}$C]-arachidonic acid (specific activity 54 Ci mmole$^{-1}$); [$^3$H]-$PGE_2$ (specific activity 59 Ci mmole$^{-1}$) and [$^3$H]-$PGF_{2\alpha}$ (specific activity 59 Ci mmole$^{-1}$) from the Radiochemical Centre, Amersham; silicic acid, 325 mesh, from Sigma Chemicals, London; 0.25 mm silicic acid TLC plates (20 x 20 cm) from Merck.

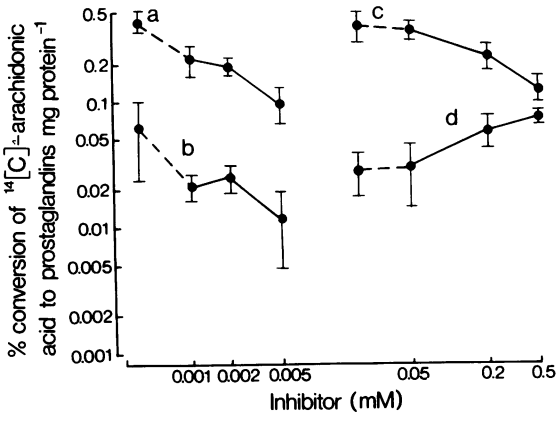


**Figure 1** Effects of metyrapone and indomethacin on prostaglandin synthesis in the pregnant rat uterus *in vitro*. (a) and (b) show the effect of indomethacin on prostaglandin $E_2$ and prostaglandin $F_{2\alpha}$ synthesis respectively; (c) and (d) show the effect of metyrapone on prostaglandin $E_2$ and prostaglandin $F_{2\alpha}$ synthesis respectively. Mean control values for each dose-response curve are shown (± s.e. mean) connected by dotted lines. The results with indomethacin were obtained from 4 different uterine homogenates and the results with metyrapone from 3 different homogenates. Using smaller amounts of added [$^{14}$C]-arachidonic acid the actions of indomethacin were confirmed with a further 2 homogenates and the actions of metyrapone with a further 3 homogenates.

## Results

*Effect of metyrapone on prostaglandin F release and uterine activity* in vitro

In rats treated with 150 mg/kg metyrapone on days 20 and 21 of pregnancy the mean prostaglandin F release over a 1 h period from single uterine horns *in vitro* determined on day 22 was 173.8 ± 21.2 (s.e mean) ng $g^{-1}$ $h^{-1}$ (7 horns). This was significantly less than the prostaglandin F release from control horns (253 ± 35.5 ng $g^{-1}$ $h^{-1}$; 6 horns) ($P < 0.05$; Wilcoxon Test). Spontaneous contractions of the control horns were well maintained through the 1 h period *in vitro*, but in horns from metyrapone-treated rats activity declined after 30 min *in vitro*.

*Validation of prostaglandin synthetase assay*

Column chromatography of control incubations of [$^{14}$C]-arachidonic acid with pregnant rat uterine homogenates followed by thin layer chromatography demonstrated that the majority of the radioactivity recovered was distributed as follows: arachidonic acid and neutral lipids in the first fraction, prostaglandin $E_2$ in the second fraction and prostaglandin $F_{2\alpha}$ in the third fraction. Nine to ten percent of the radioactivity originally added to control incubates as [$^{14}$C]-arachidonic acid was converted to prostaglandins. In one experiment pellets from centrifugation and all aqueous layers were counted and the remaining 90% of the radioactivity was accounted for as unchanged arachidonic acid, label taken up by tissue (probably into phospholipids) and water-soluble products. Recovery of [$^{3}$H]-prostaglandin $E_2$ (15,000 d/min) through extraction and chromatography was 65-70% and recovery of [$^{3}$H]-prostaglandin $F_{2\alpha}$ (60,000 d/min) was 55-65%.

*Effects of metyrapone and indomethacin on prostaglandin synthesis* in vitro

Metyrapone introduced *in vitro* inhibited prostaglandin $E_2$ synthesis in pregnant rat uterine homogenates in a dose-dependent manner. Indomethacin inhibited both prostaglandin $E_2$ synthesis (about 150 times more effectively than metyrapone) and prostaglandin $F_{2\alpha}$ synthesis (Figure 1).

## Discussion

The results presented here show that metyrapone, an inhibitor of the 11β-hydroxylation step in the biosynthesis of corticosteroids (Chart & Sheppard, 1959) is also an inhibitor of prostaglandin synthesis, but has differential effects *in vivo* and *in vitro*. In the isolated pregnant rat uterus prostaglandin $F_{2\alpha}$ is the predominant prostaglandin released (Vane & Williams, 1973). This observation was confirmed in one experiment using column chromatography and bioassay against authentic prostaglandins. In this preparation inhibition of prostaglandin $F_{2\alpha}$ release from the uterus was observed after administration of metyrapone to pregnant rats. However, in homogenates prepared from pregnant rat uteri, prostaglandin $E_2$ was the predominant prostaglandin produced and the addition of metyrapone to the homogenate resulted in inhibition of prostaglandin $E_2$ synthesis but a paradoxical stimulation of prostaglandin $F_{2\alpha}$ synthesis. This effect of metyrapone was observed over a wide range of metyrapone concentrations and contrasted with indomethacin which inhibited the synthesis of both prostaglandins. We are unable to offer an explanation for the observation that the isolated uterus produces predominantly

prostaglandin F when homogenates produce predominantly prostaglandin E. Preliminary experiments have shown that the balance of prostaglandin E and prostaglandin F synthesis in homogenates is unaffected by altering either substrate concentration or incubation time. However, it is pertinent to note that other workers have reported differential effects of inhibitors on prostaglandin synthesis, which are related to changes in conditions such as substrate concentration (Flower, Cheung & Cushman, 1973) or cofactors (Maddox, 1973). Similarly, the effect of metyrapone on prostaglandin synthesis in uterine homogenates may be sensitive to changes in experimental conditions. It is of interest to note that the structure of metyrapone (2-methyl-1,2-bis-(3-pyridyl)-1-propanone) is similar to those of many other prostaglandin synthetase inhibitors which also consist of two interconnected ring systems (Gryglewski, 1974).

Inhibition of prostaglandin synthesis may explain the prolongation of pregnancy produced by metyrapone in the rat (Parvez *et al.*, 1972), since several prostaglandin synthetase inhibitors have been shown to prolong and/or delay parturition in this species (see Aiken, 1974). It is unlikely that metyrapone affects parturition through inhibition of foetal adrenocorticosteroidogenesis, as there is little evidence for foetal involvement in parturition in the rat (Jost, 1973). However, ovarian oestrogen is implicated in parturition in the rat (Yoshinaga, Hawkins & Stocker, 1969) and as metyrapone inhibits oestrogen synthesis (Griffiths, 1963; Matsumoto, Kotoh, Miyata & Kurachi, 1965) this may, in part, explain its effects on parturition.

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## References

AIKEN, J.W. (1972). Aspirin and indomethacin prolong parturition in rats: evidence that prostaglandins contribute to expulsion of foetus. *Nature, Lond.*, **240**, 21-25.

AIKEN, J.W. (1974). Prostaglandins and prostaglandin synthetase inhibitors; Studies on uterine motility and function. In *Prostaglandin Synthetase Inhibitors – Their effects on physiological functions and pathological states*, ed. Robinson, H.J. & Vane, J.R., pp. 289-302, New York: Raven Press.

BLACKWELL, G.J., FLOWER, R.J., PARSONS, M.F. & VANE, J.R. (1975). Factors influencing the turnover of prostaglandin synthetase. *Br. J. Pharmac.*, **53**, 467P-468P.

CHART, J.J. & SHEPPARD, H. (1959). Pharmacology and biochemistry of some amphenone analogues and other adrenal cortical inhibitors. *J. Med. Pharm. Chem.*, **1**, 407-441.

CHESTER, R., DUKES, M. SLATER, S.R. & WALPOLE, A.L. (1972). Delay of parturition in the rat by anti-inflammatory agents which inhibit the biosynthesis of prostaglandins. *Nature, Lond.*, **240**, 37-38.

COHEN, A. (1973). Plasma corticosterone concentration in the foetal rat. *Horm. Metab. Res.*, **5**, 66.

DUNN, M.V., HUMPHRIES, N.G., JUDKINS, G.R., KENDALL, J.S. & KNIGHT, G.W. (1973). The effect of prostaglandin $F_{2\alpha}$ antibody on gestation length in the rat. *Prostaglandins*, **3**, 509-514.

DUPOUY, J-P. (1972). Activité corticostimulante de l'hypophyse du foetus de Rat après surrénalectomie maternelle et injection de métopirone à différents stades de la gestation. *C.R. Acad. Sc. Paris*, **274**, Série D, 3585-3588.

FLOWER, R.J., CHEUNG, H.S. & CUSHMAN, D.W. (1973). Quantitative determination of prostaglandins and malonaldehyde formed by the arachidonate oxygenase (prostaglandin synthetase) system of bovine seminal vesicle. *Prostaglandins*, **4**, 325-342.

GRIFFITHS, K. (1963). Inhibition of '19-hydroxylase' activity in the golden hamster adrenal by SU 4885. *J. Endocr.*, **26**, 445-446.

GRYGLEWSKI, R.J. (1974). Structure-activity relationships of some prostaglandin synthetase inhibitors. In *Prostaglandin Synthetase Inhibitors – Their effects on physiological functions and pathological states*, ed. Robinson, H.J. & Vane, J.R., pp. 33-52, New York: Raven Press.

HARNEY, P.J., SNEDDON, J.M. & WILLIAMS, K.I. (1974). The influence of ovarian hormones upon the motility and prostaglandin production of the pregnant rat uterus *in vitro*. *J. Endocr.*, **60**, 343-351.

JOST, A. (1973). Does the foetal hypophyseal-adrenal system participate in delivery in rats and in rabbits? In *Foetal and Neonatal Physiology*, ed. Comline, K.S., Cross, K.W., Dawes, G.S. & Nathanielsz, P.W., pp. 589-593, London: Cambridge University Press.

KALAVSKY, M. (1971). Foetal rat adrenal steroidogenesis. *Biol. Neonat.*, **17**, 427-435.

KIRSCH, R.E. (1938). A study on the control of length of gestation of the rat with notes on maintenance and termination of pregnancy. *Amer. J. Physiol.*, **122**, 86-93.

LIGGINS, G.C., FAIRCLOUGH, R.J., GRIEVES, S.A., KENDALL, J.Z. & KNOX, B.S. (1973). The mechanism of initiation of parturition in the ewe. *Rec. Prog. Horm. Res.*, **29**, 111-150.

LOWRY, O.H., ROSEBROUGH, N.J., FARR, A.L. & RANDALL, R.J. (1951). Protein measurement with the Folin phenol reagent. *J. biol. Chem.*, **193**, 265-275.

MADDOX, I.S. (1973). Copper in prostaglandin synthesis. *Biochim. Biophys. Acta*, **306**, 74-81.

MATSUMOTO, K., KOTOH, K., MIYATA, J. &

KURACHI, K. (1965). Effect of 2-methyl-1,2-bis-(3-pyridyl)-1-propanone (SU 4885) on oestrogen biosynthesis *in vivo*. *J. Endocr.*, **33**, 317-318.

NUGTEREN, D.H. & HAZELHOF, E. (1973). Isolation and properties of intermediates in prostaglandin biosynthesis. *Biochem. Biophys. Acta*, **326**, 448-461.

PARNHAM, M.J. & SNEDDON, J.M. (1975). Metyrapone inhibits prostaglandin synthesis and release from the pregnant rat uterus *in vitro*. *Br. J. Pharmac.*, **54**, 278P.

PARNHAM, M.J., SNEDDON, J.M. & WILLIAMS, K.I. (1975). Evidence for a possible foetal control of prostaglandin release from the pregnant rat uterus *in vitro*. *J. Endocr.*, **65**, 429-437.

PARVEZ, S., PARVEZ, H. & ROFFI, J. (1972). Gestation prolongée chez des rattes traitée par la Métopirone. *C.R. Acad. Sc. Paris*, **274**, *Série D*, 262-263.

SELYE, H., COLLIP, J.B. & THOMSON, D.L. (1935). Endocrine interrelations during pregnancy. *Endocrinology*, **19**, 151-159.

SWAAB, D.F. & HONNEBIER, W.J. (1973). The influence of the fetal rat brain upon the intrauterine growth of the fetus and the placenta and on gestation length. *J. Obstet. Gynaec. Br. Common.*, **80**, 589-597.

VANE, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature, New Biol.*, **231**, 232-235.

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

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

YOSHINAGA, K., HAWKINS, R.A. & STOCKER, J.F. (1969). Estrogen secretion by the rat ovary *in vivo* during the oestrus cycle and pregnancy. *Endocrinology*, **85**, 103-112.

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