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The contribution of prostaglandin production to contractions of the isolated uterus of the rat

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Summary

1. Indomethacin and meclofenamate, both of which are potent inhibitors of prostaglandin synthetase, antagonized the contractor effects of oxytocin on the isolated uterus from the non-pregnant rat. Contractions induced by acetylcholine or prostaglandin $F_{2\alpha}$ were not antagonized.

2. Uteri from rats 17–22 days pregnant exhibited intermittent spontaneous contractions when used as isolated preparations. They also released prostaglandin-like activity (mainly similar to $F_{2\alpha}$) into the bathing fluid. Both the prostaglandin release and the uterine activity were abolished by indomethacin. Activity could be restored by addition of low concentrations of prostaglandin E_2 or $F_{2\alpha}$.

3. The release of prostaglandin $F_{2\alpha}$-like activity by the uteri increased dramatically on the expected day of delivery (day 22).

4. The results add force to the hypothesis that the spontaneous activity of some isolated organs is due to an intramural prostaglandin generation, and that increased uterine prostaglandin production contributes to the expulsion of the foetus.

Introduction

Prostaglandins of the E and F series have been implicated in uterine function by several observations. They are highly potent in causing uterine contraction in several species (see Bergström, Carlson & Weeks, 1968) and are released by distension of the guinea-pig uterus (Poyser, Horton, Thompson & Los, 1971). The increases in prostaglandin concentrations in human blood (Karim, 1968) and amniotic fluid (Karim & Devlin, 1967) during labour suggest that they may play a part in the expulsion of the uterine contents at parturition. Recent work by Aiken (1972) and Chester, Dukes, Slater & Walpole (1972) confirms this suggestion by showing that parturition is delayed and prolonged in rats when prostaglandin production is prevented by prostaglandin synthetase inhibitors such as aspirin and indomethacin.

We have used indomethacin and meclofenamate, both potent inhibitors of prostaglandin biosynthesis (Flower, Gryglewski, Herbaczynska-Cedro & Vane, 1972) to study the contribution of intramural prostaglandin production to spontaneous and drug-induced contractions of the rat isolated uterus. Contractions of the non-pregnant isolated uterus from the rat elicited by oxytocin were

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antagonized by indomethacin whereas those evoked by $PGF_{2\alpha}$ and acetylcholine were not. Spontaneous contractions of pregnant rat isolated uteri were also blocked by indomethacin and there was a consistent decline in prostaglandin output. Oxytocin-induced contractions of pregnant uteri were not antagonized by indomethacin.

Some of these results have been communicated to the British Pharmacological Society (Vane & Williams, 1972; Williams, 1973).

Methods

Non-pregnant uteri

Virgin Wistar rats weighing 150–250 g were killed and the uteri removed. The stage of oestrus was not noted. Each uterine horn was mounted in a 15 ml organ bath and bathed in Garcia de Jalón solution (1947) at 34° C. Isotonic contractions against a load of 1 g were recorded on a pen recorder. Drugs were added to the bath at 4 min intervals and left in contact with the preparation for 30–90 seconds. Other preparations were superfused (Gaddum, 1953) at 10 ml/min with Krebs solution at 37° C. Drugs were infused for 1–3 min and contractions against an initial load of 1 g were measured by an auxotonic lever (Paton, 1957) and displayed on a pen recorder.

Pregnant uteri

Female Wistar rats were mated separately and the finding of a cervical plug was denoted day 1 of pregnancy. Rats 17–22 days pregnant were used. Uteri were removed, each horn opened longitudinally and the contents removed. Whole uteri or single horns were mounted in 15 ml organ baths and bathed with Krebs solution at 37° C. Isotonic uterine contractions against a load of 2 g were recorded. Bath fluid was withdrawn at 15 min intervals for extraction and the bath washed by overflow.

Duck pulmonary vein

This tissue, which is contracted by oxytocin, was prepared according to Vane & Williams (1970).

Extraction, separation and identification of prostaglandins

Bath fluid was acidified to pH3 with 1N hydrochloric acid and extracted twice with one volume of ethyl acetate. This aqueous phase was discarded. The ethyl acetate phases were pooled and evaporated to dryness *in vacuo* at 40° C. The residue was stored at −12° C until required for assay.

Residues were taken up in 2 ml of Krebs solution and assayed for prostaglandin-like activity on a rat stomach strip, rat colon and chick rectum superfused with Krebs solution (6–8 ml/min) containing a mixture of antagonists (Gilmore, Vane & Wyllie, 1968). Test samples and standard prostaglandins were injected in a fixed volume of 0·5 ml.

Extracts for thin-layer chromatography were taken up in 0·5 ml of absolute ethanol and applied (alongside standard prostaglandins) to glass plates pre-coated with 0·25 mm of silica gel (Merck) with a disposable micro-pipette (Microcap:

Drummond). Plates were developed in the AI solvent system (Gréen & Samuelsson, 1964). The positions of the authentic prostaglandins were visualized and the corresponding parts of the bath fluid sample regions, as well as the intermediate zones, were scraped off the plate and taken up with Krebs solution. Samples were then assayed as above.

Gas chromatography

Evaporated extracts of bath fluid were reconstituted in 100 μl of methanol and treated sequentially as follows:

(a) *Methylation.* The sample was treated with 100 μl of diazomethane in ether for 15 min at 18° C. Excess diazomethane was blown off with nitrogen.

(b) *Methoximation.* 100 μl of pyridine was added to the methyl esters which were then treated with 100 μl of methoxamine in pyridine (1:1) for 6 h at 18° C. The residue was again dried with nitrogen.

(c) *Acetylation.* The sample was reacted with 200 μl of acetic anhydride in pyridine (1:1) for 18 h at 18° C and stored in a vacuum desiccator overnight. The residue was taken up in 100 μl of hexane or methylene chloride and 1–10 μl samples injected onto the column.

Derivatives were similarly prepared from 500 ng samples of authentic prostaglandin E_2 and $F_{2\alpha}$.

The samples were analysed on a Pye 104 Gas chromatograph. The column (1·5 m×5 mm i.d.) was packed with 1·2% SE 30 on Chromosorb W. Column temperature was 200–232° C and the carrier gas, nitrogen, flowed at 2 litres/minute. A flame ionization detector was used. The retention times on the column for derivatives of authentic prostaglandins were compared with those for experimental samples.

The salt solutions used had the following composition (in g/litre of distilled water): Krebs bicarbonate solution: NaCl, 6·9; KCl, 0·35; $CaCl_2$, 0·55 KH_2PO_4, 0·16; $MgSO_4$ $7H_2O$, 0·29; glucose, 1·0; $NaHCO_3$, 2·1; Garcia de Jalon solution: NaCl, 9·0; KCl, 0·42; $CaCl_2$, 0·12; $MgSO_4$ $7H_2O$, 0·38; glucose, 1·0; $NaHCO_3$, 0·5.

Drugs

The following drugs were used, doses of salts are expressed in terms of the base: acetylcholine chloride (Sigma); indomethacin (Merck, Sharp & Dohme); meclofenamic acid (Parke Davis); oxytocin (Pitocin; Parke Davis); prostaglandins E_2 and $F_{2\alpha}$ (Upjohn).

Results

Drug-induced contractions

Figure 1 shows log dose-response curves for oxytocin, prostaglandin $F_{2\alpha}$ and acetylcholine, constructed from two experiments with rat isolated uteri. The upper curves show that the action of oxytocin was antagonized by meclofenamate; a concentration of 2·8 μM gave a dose ratio of 1·7 and 5·6 μM a dose ratio of 4. The effects of prostaglandin $F_{2\alpha}$ and acetylcholine were unchanged. The lower

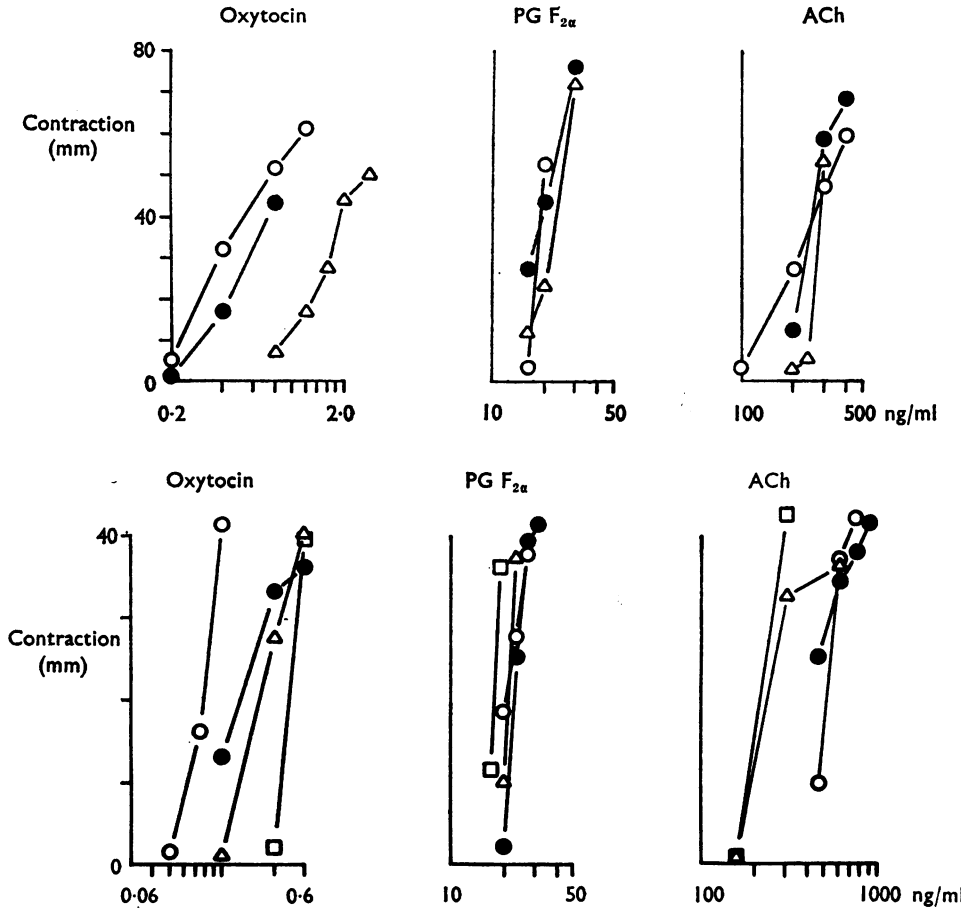

FIG. 1. Prostaglandin production contributes to contractions of the rat isolated uterus elicited by oxytocin. Graphs show the log dose-response curves to oxytocin, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and acetylcholine (ACh) in two experiments. The curves are control (○) or in the presence of 2·8 (●), 5·6 (△), or 22·4 μM (□) meclofenamate (top) or indomethacin (bottom).

part of the figure shows that similar results were obtained with indomethacin, increasing concentrations producing increasing antagonism of oxytocin (dose-ratio of 1·8, 2·2 and 3·3 in the presence of 2·8, 5·6 and 22·4 μM indomethacin respectively). The effects of prostaglandin $F_{2\alpha}$ were unchanged and those of acetylcholine were potentiated by indomethacin. However, potentiation was not generally observed.

In 18 experiments indomethacin (0·7–2·8 μM) antagonized the effects of oxytocin to give a dose-ratio of 5 ± 1 (mean $\pm$ S.E. of mean) whereas the effects of prostaglandin $F_{2\alpha}$ were relatively unchanged (dose-ratio $1{\cdot}6 \pm 0{\cdot}5$; 12 experiments).

However, in uteri from pregnant rats, drug-induced contractions were not antagonized. In only two of ten experiments did indomethacin (2·8–11·2 μM) reduce the magnitude and duration of contractions induced by oxytocin 100–3,200 μu/ml).

Strips of duck pulmonary vein were also used, to find whether the effects of oxytocin on another tissue were antagonized. Indomethacin (2·8–11·2 μM ; 3

experiments, had no inhibitory effect on contractions induced by either oxytocin or adrenaline (Figure 2).

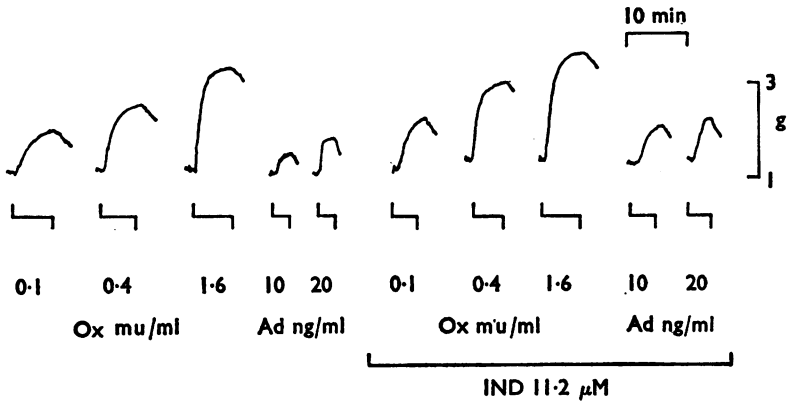

FIG. 2. Isometric contractions of the duck pulmonary vein are not affected by indomethacin. The tracing shows the contractions of a duck pulmonary vein induced by oxytocin (Ox) or adrenaline (Ad). Inclusion of indomethacin (IND) 11·2 μM did not reduce the responses. Time: 10 min; vertical scale: 1–3 g tension.

Spontaneous contractions

In fifteen experiments the spontaneous contractions of uterine horns isolated from rats 17–22 days pregnant were abolished by indomethacin or meclofenamate. Figure 3 shows such an experiment, in which two uterine horns from a 21 day pregnant rat were used separately. Indomethacin (5·6 μM) added to one horn

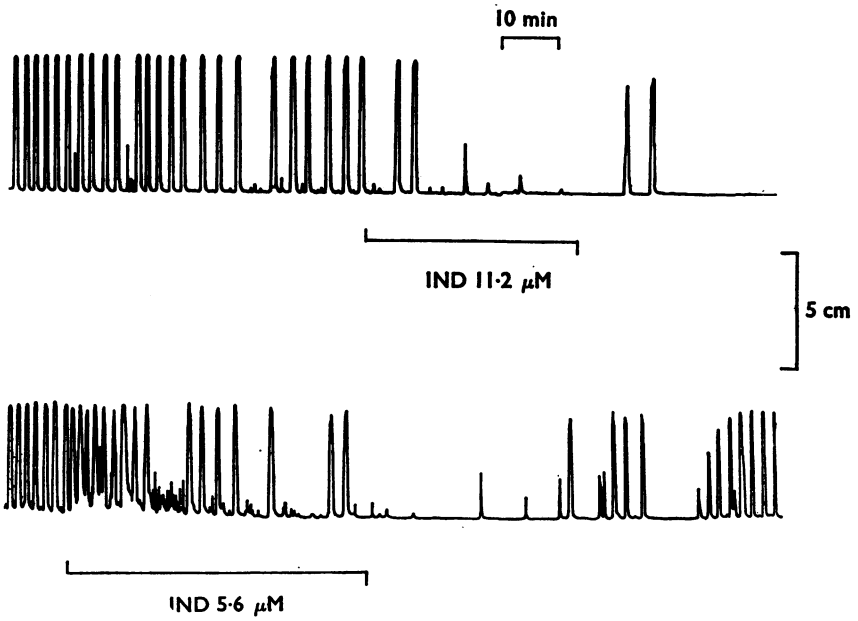

FIG. 3. Prostaglandin production contributes to the spontaneous contractions of the pregnant rat uterus *in vitro*. Records show the isotonic contractions of two uterine horns from a rat 21 days pregnant. Inclusion of indomethacin (IND) 5·6 μM in the fluid bathing the lower tissue slowly reduced spontaneous activity. Indomethacin (11·2 μM) led to a faster decline of spontaneous contractions of the upper preparation. Time: 10 min; vertical scale: 5 cm.

(lower trace) led to a gradual decline in the frequency of the contractions. The effect of the drug persisted after washing out; spontaneous activity was completely abolished and only returned to control frequency after sixty minutes. There was little change in the frequency of contraction of the other horn until indomethacin (11·2 μM) was added to the bathing fluid. This induced a rapid decline in frequency of contraction and the preparation soon became quiescent. Indomethacin treatment was discontinued, but uterine activity did not return during the remainder of the experiment.

Prostaglandin release from pregnant uteri

Extracts of samples of the fluid bathing uteri from pregnant rats contained prostaglandin-like activity. This did not exactly match prostaglandin E_2 or $F_{2\alpha}$ in that several of the extracts caused relaxation of the chick rectum, an effect only rarely seen and to a lesser extent with $F_{2\alpha}$. Thin layer chromatography of the extracts in the AI solvent system confirmed the presence of E and F-type prostaglandins. Figure 4 shows the results obtained with one extract; the major part of the activity was in the zone equivalent to prostaglandin $F_{2\alpha}$. Some activity was also present in the zone corresponding to prostaglandin E_2 and in the intermediate zone. No prostaglandin-like activity was present in other zones of the plate.

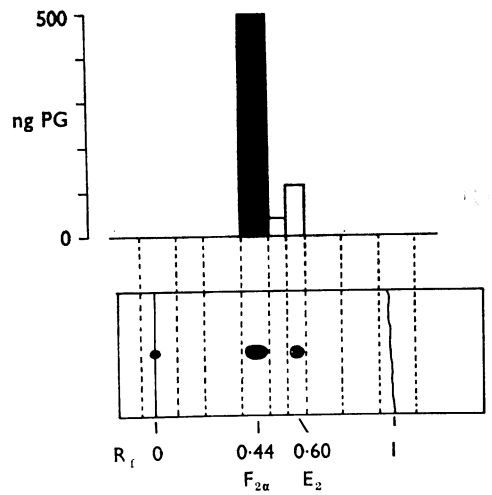

FIG. 4. Thin layer chromatography (AI system) of an extract of fluid which had bathed a 22 day pregnant uterus. The lower portion of the figure shows the mobility of authentic prostaglandin E_2 and $F_{2\alpha}$. The histogram illustrates the localization of prostaglandin (PG)-like activity in the corresponding test zones of the plate assayed as prostaglandin $F_{2\alpha}$ (■) or E_2 (□). No other activity was found.

Gas chromatography of samples gave peaks whose retention times corresponded to those for the methyl esters of palmitic and stearic acid (1·8 and 3·8 minutes). Peaks also occurred whose retention times were similar to those for the methyl/acetyl derivative of authentic prostaglandin $F_{2\alpha}$ (3·9 minutes). No peak was noted which corresponded to the methyl/methoxime/acetyl derivative of prostaglandin E_2 (3·2 min); the minimal detectable dose of this compound was 50 ng.

Figure 5 shows the amount of prostaglandin-like material (assayed as $F_{2\alpha}$) released by uteri taken from rats in the last few days of pregnancy. Uteri taken from animals on the day of delivery (day 22), but before parturition had started, released much more prostaglandin-like activity than did those taken at days 19–21.

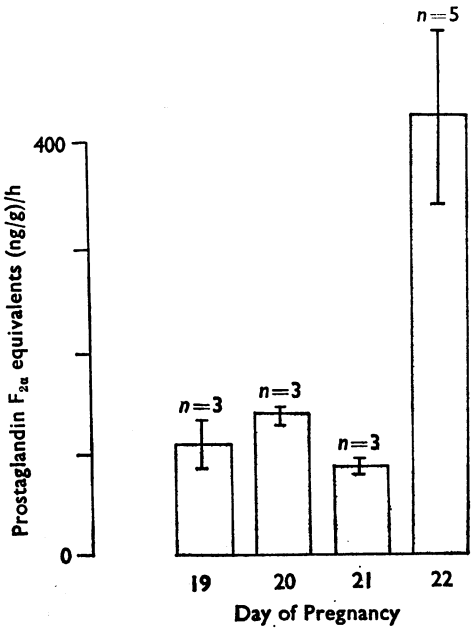

FIG. 5. Variation in prostaglandin release from the rat uterus *in vitro* with the duration of pregnancy. Columns show the mean amounts of prostaglandin-like activity (assayed as prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)) released into the bathing fluid over a one hour period by uteri removed from rats on the 19–22 days of pregnancy. Vertical lines show standard errors.

Relationship between prostaglandin production and uterine contraction

To integrate the activity of the uteri from pregnant rats, the total area beneath the contractions was calculated for consecutive 15 min periods. The number obtained for the first period was taken as 100% activity, so that those for subsequent periods could be expressed as a percentage. Figure 6 shows that indomethacin (11·2 μM) reduced both contractile activity and output of prostaglandin-like material from a 21 day pregnant rat uterus. In contrast, the control preparation maintained consistent muscle activity throughout the experiment and release of prostaglandin-like material increased somewhat towards the end of the experiment. Similar results were obtained in six other experiments.

Addition of prostaglandin E_2 or $F_{2\alpha}$ to the bathing fluid restored intermittent contractions to uteri rendered quiescent by indomethacin (Figure 7). Prostaglandin E_2 was more potent than $F_{2\alpha}$ (3 experiments). Neither prostaglandin increased the resting tone of the uterus.

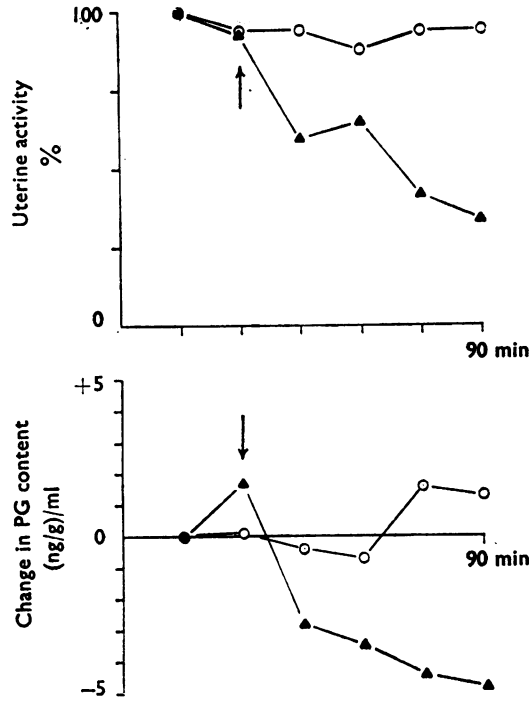

FIG. 6. Relationship between spontaneous activity of the pregnant rat uterus *in vitro* and prostaglandin release. The upper graph shows the activity of control (○) and test (▲) uteri taken from 21 day pregnant rats. The lower graph shows the changes in prostaglandin output from these tissues. At the arrows, indomethacin (11·2 μM) was included in the fluid bathing the test preparation and was then present throughout. The prostaglandin-like activity fell to undetectable concentrations.

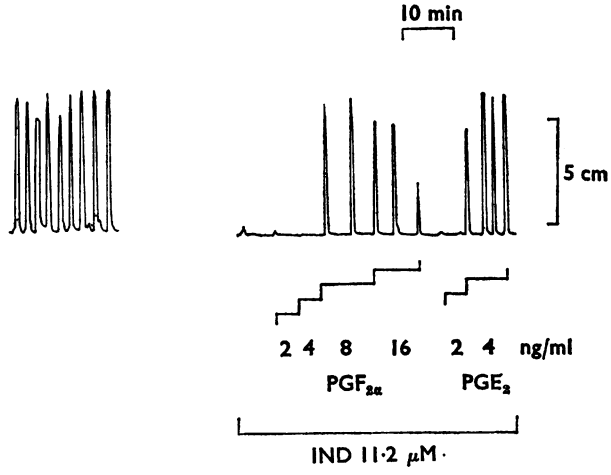

FIG. 7. Prostaglandins restore activity to the pregnant rat uterus rendered quiescent with indomethacin. Spontaneous contractions of a uterine horn from a rat 19 days pregnant were abolished by indomethacin (IND) 11·2 μM. After 45 min the effects of adding prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) or E_2 cumulatively to the bath were tested. Time : 10 min; vertical scale: 5 cm.

Discussion

In isolated uteri from non-pregnant rats, indomethacin and meclofenamate antagonized the contractor activity of oxytocin, but not that of acetylcholine or prostaglandin $F_{2\alpha}$. The antagonism was seen with low concentrations of the anti-inflammatory drugs, within the range known to inhibit prostaglandin biosynthesis in other tissues (Vane, 1971; 1972; Ferreira, Moncada & Vane, 1971; Smith & Willis, 1971; Flower *et al.*, 1972). The antagonism was unlikely to be due to an unrelated non-specific action of these drugs; for instance, on the oxytocin receptor, because no antagonism was seen on the duck pulmonary vein, or even on uteri from pregnant rats.

Inhibition of prostaglandin production in rat uteri might reduce the effects of oxytocin in at least two ways. Firstly, there may be a continuous prostaglandin synthesis which sensitizes the uterus to the action of oxytocin. Prostaglandins potentiate responses to oxytocin in the rat isolated uterus and in strips of human pregnant myometrium (Pickles, Hall, Clegg and Sullivan, 1966; Brummer, 1972). However, such potentiation is non-specific and would not explain why in our experiments only oxytocin-induced responses were inhibited by indomethacin. Furthermore, using bioassay, we have no evidence for a basal release of prostaglandin from the non-pregnant uterus and indeed, had it been present, spontaneous contractions should also have occurred.

A second possibility is that oxytocin (but not acetylcholine or prostaglandin $F_{2\alpha}$) as well as inducing muscle contraction, stimulates a synthesis of prostaglandins. The prostaglandins so produced would then potentiate the contraction induced by oxytocin, perhaps by increasing the rate and spread of depolarization as suggested by Clegg, Hall & Pickles (1966). Certainly, in other tissues, peptides such as angiotensin and bradykinin can cause prostaglandin release (McGiff, Crowshaw, Terragno & Lonigro, 1970; McGiff, Terragno, Malik & Lonigro, 1972; Aiken & Vane, 1971; 1973; Ferreira, Moncada & Vane, 1973). If the capacity of the uterus to synthesize prostaglandins increases towards the end of pregnancy (see below) this would help to explain the progressive increase in sensitivity of the pregnant human uterus to oxytocin (Caldeyro-Barcia & Sereno, 1961; Brummer, 1971). However, our failure to modify the action of oxytocin on the pregnant rat uterus with indomethacin would suggest that such an effect is not attributable to a gradual and progressive change in uterine prostaglandin production. Although in our experiments, indomethacin reduced prostaglandin output to amounts undetectable by bioassay, potentiation of agonist action can occur with very small amounts of prostaglandin (Pickles *et al.*, 1966; Brummer, 1972) and thus complete inhibition of prostaglandin synthesis may be necessary in order to affect the oxytocin response.

Isolated uteri from pregnant rats release prostaglandins into the bathing fluid and exhibit spontaneous contractions; both of these properties are abolished by indomethacin. Thus, as in other isolated smooth muscles such as the cat iris (Posner, 1970), rabbit jejunum (Ferreira, Herman & Vane, 1972) rat stomach strip and guinea-pig colon (Eckenfels & Vane, 1972) a local prostaglandin production maintains the inherent smooth muscle activity. For the pregnant rat isolated uterus Aiken (1972) has come to a similar conclusion; he further showed that papaverine inhibits spontaneous activity without affecting prostaglandin production, thus eliminating the possibility that prostaglandin release is a consequence of

uterine contraction. Since generation of prostaglandins can be initiated by mechanical stimuli such as touch or damage (Piper & Vane, 1971), the prostaglandin output from isolated uteri of pregnant rats could be a result of the stress of isolating the uterus, removing the contents and bathing it in an artificial salt solution. However, our *in vitro* findings were supported by the results of Aiken (1972) and Chester *et al.* (1972) who found that indomethacin and similar drugs delayed and prolonged parturition in rats. They concluded that prostaglandin production by the uterus is necessary for foetal expulsion at term. Further evidence for the participation of uterine prostaglandins in parturition was the dramatic increase in prostaglandin $F_{2\alpha}$ production near term, at a time when the frequency of uterine contraction is known to increase; Aiken (1972) obtained similar results with uteri taken from rats during parturition. The endometrium appears to be the sole source of prostaglandins in the pregnant rat uterus (Williams, 1973) and the preliminary experiments of Challis, Harrison, Heap, Horton & Poyser (1972) indicate that prostaglandin output by the pregnant uterus of the sheep may be under hormonal control. Our findings certainly suggest that the increased ability of the uterus to produce prostaglandins precedes, and may well play a role, in the initiation of parturition.

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