Br. J. Pharmac. (1974), 52, 393-399

EFFECTS OF PROSTAGLANDIN INHIBITORS ON ANGIOTENSIN, OXYTOCIN AND PROSTAGLANDIN $F_{2\alpha}$ CONTRACTILE EFFECTS ON THE RAT UTERUS DURING THE OESTROUS CYCLE

MARYVONNE BAUDOUIN-LEGROS, P. MEYER & M. WORCEL

Unite de Recherches de Physiologie et
Pharmacologie Vasculaire et Renale. INSERM U7, Hôpital Necker, 161, rue de Sèvres, 75015 Paris, France

1 In the rat isolated uterus maximal spontaneous contractions and maximal sensitivity to angiotensin II, oxytocin and prostaglandin $F_{2\alpha}$ were observed in di- and proestrus. Minimal sensitivity to the three agonists was observed in metoestrus. Maximal contractile effects of angiotensin II, oxytocin and prostaglandin $F_{2\alpha}$ were thus observed when the ratio oestrogen/progesterone levels was high.

2 The oestrogen-dependent sensitivity of the rat uterus is partially mediated by endogenous prostaglandins. Indomethacin suppressed the increased sensitivity to angiotensin and oxytocin present in dioestrus and proestrus but did not affect that to prostaglandin $F_{2\alpha}$. Polyphloretin phosphate at a concentration of 10 μg/ml resulted in complete identity of dioestrus and metoestrus dose-response curves to angiotensin and oxytocin.

3 Spontaneous uterine contractions observed when oestrogen levels are high are also dependent on intramural prostaglandins as they were inhibited by indomethacin and polyphloretin phosphate. In the metoestrus uterus, prostaglandin $F_{2\alpha}$ induced the reappearance of spontaneous contractions.

4 Prostaglandin $F_{2\alpha}$ had a potentiating effect on angiotensin-elicited contractions which persisted after washing out prostaglandin $F_{2\alpha}$.

Introduction

Oestrogens probably influence uterine responses to catecholamines as, after oestrogen administration (Diamond & Brady, 1966; Tothill, 1967; Paton, 1968), during natural oestrus (Butterworth & Randall, 1970), in late pregnancy (Tothill, 1967) and in the puerperium (Abdel-Aziz & Bakry, 1972), contractile responses to catecholamines are demonstrable in the rat uterus. It has been assumed that these motor effects are due to the induction of α-adrenoceptors (Tothill, 1967; Butterworth & Randall, 1970) but several investigators have shown that they are in fact related to the release of prostaglandins (Tothill, Rathbone & Willman, 1971; Aiken, 1972; Vane & Williams, 1972).

The uterine response to oxytocin is also partially dependent upon release of prostaglandins (Vane & Williams, 1972). In addition, in late pregnancy increased oestrogen production is accompanied by a marked enhancement of uterine prostaglandin synthesis which is maximal near term, at a time where the frequency of uterine contraction is known to increase (Vane & Williams, 1973).

The aim of the present study was to investigate the hypothesis that steroid-dependent variation in uterine contractility could be mediated by fluctuations of prostaglandin synthesis or release. The action of prostaglandin inhibitors on the effect of various oxytocic agents was therefore studied.

Methods

Virgin Wistar rats weighing 200-225 g were used in these experiments. The different phases of the oestrous cycle were determined by the examination of vaginal smears, collected in duplicate from each animal and studied after May-Grünwald Giemsa fixation according to Slider & Downey (1950). The rats were killed by a blow on the head and the uterus removed. Each uterine horn was mounted in a 10 ml organ bath filled with Krebs solution at 37°C, and attached to an isotonic lever

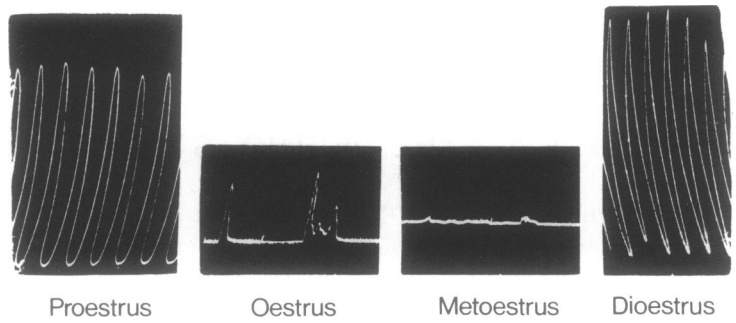

Fig. 1 Spontaneous contractions of rat isolated uterus in different stages of the oestrous cycle. The metoestrous uterus often exhibited no contractions at all. In dioestrus and proestrus the contractions were strong and regular.

providing a 4 g load and a 4-fold magnification. Contractions were recorded on a smoked drum. The Krebs solution had the following composition (mM): NaCl 111.2, KCl 4.7, $CaCl_2$ 1.5, KH_2PO_4 1.2, $MgSO_4$ 2.4, $NaHCO_3$ 10.5, NaH_2PO_4 0.9, Na_2HPO_4 2.3, glucose 10.0. The solution was bubbled with 95% O_2 and 5% CO_2 (pH 7.4). The organs were equilibrated in the solution for at least 90 minutes. Drugs were dissolved in the same solution, introduced in the bath in a volume of less than 100 μl, and left in contact with the organ until the highest contraction was obtained. The drug was then removed by washing the preparation with fresh Krebs solution. Successive drug doses were applied at 15 min intervals and consecutive dose-response curves were separated by a period of 90 minutes. Antagonists were allowed to act for 30 min before testing the activity of the agonists, and were left in the bathing solution from then on. Doses of agonists were not randomized.

Drugs

The following drugs were used: (1-Asn, 5-Val)-angiotension II (Hypertensin, Ciba); oxytocin (Syntocinon, Sandoz); prostaglandin $F_{2\alpha}$ (Upjohn); indomethacin (Merck, Sharp and Dohme); polyphloretin phosphate (Leo).

Results

Spontaneous uterine contractility during the oestrous cycle

Spontaneous contractions of the rat uterus vary during the oestrous cycle. In dioestrus and proestrus contractions are strong and regular, whereas they are weak and irregular in oestrus. Spontaneous contractions almost disappear in metoestrus (Figure 1).

Indomethacin was added to the bath at a concentration of 5×10^{-6} M, which seems to inhibit specifically prostaglandin synthesis in tissue homogenates (Vane, 1971). Indomethacin very rapidly suppressed the spontaneous contractions observed in proestrus and dioestrus. The contractions disappeared completely after less than 1 min exposure to the drug. This indomethacin effect was reversible, the spontaneous rhythm reappearing 30 min after removal of the drug.

Polyphloretin phosphate (PPP) which has been reported to be a specific inhibitor of prostaglandins in other organs (Eakins, Karim & Miller, 1970), completely suppressed the spontaneous contractions of the proestrus and dioestrus uterus after 1 min exposure at the concentration of 50 μg/ml. This effect persists for at least 30 min after washing out the drug. Rhythmic contractions reappeared following the administration of prostaglandin $F_{2\alpha}$ 5×10^{-6} M, even in the presence of PPP, and persisted for at least 30 min after washing out both drugs. At a concentration of 10 μg/ml, PPP decreased the amplitude and frequency of the spontaneous contractions only in pro- and dioestrus.

It is interesting to note that rhythmic contractions similar to those observed in pro- and dioestrus were induced in the normally silent metoestrus uteri after exposure to a single dose of 10^{-8} M prostaglandin $F_{2\alpha}$. The contractions persisted for at least 30 min after prostaglandin $F_{2\alpha}$ had been washed out.

Uterine response to angiotensin, oxytocin and prostaglandin $F_{2\alpha}$ during the oestrous cycle

The dose-response curves to angiotensin, oxytocin

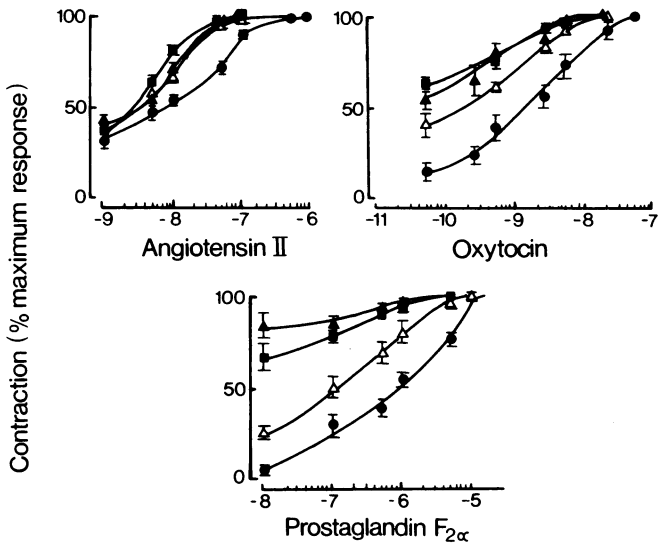

Fig. 2 Log dose-response curves to angiotensin II, oxytocin and prostaglandin $F_{2\alpha}$ in the four stages of the oestrous cycle (proestrus (■); oestrus (△); metoestrus (●); dioestrus (▲). Responses are expressed as a percentage of the maximum response. Each point is the mean from 30 experiments; vertical bars show s.e. mean.

and prostaglandin $F_{2\alpha}$ recorded throughout the oestrous cycle are shown in Figure 2. The dose-response curve to angiotensin II is significantly shifted to the right in metoestrus; the sensitivity to angiotensin is higher in the three other stages of the cycle.

More gradual variations of responses were observed with oxytocin and prostaglandin $F_{2\alpha}$. The lowest sensitivity to these agonists was in metoestrus, as in the case of angiotensin II. The sensitivity to oxytocin and prostaglandin $F_{2\alpha}$ decreased gradually in the following order: dioestrus $\geqslant$ proestrus $>$ oestrus $>$ metoestrus. During dioestrus and proestrus the uterus was sometimes sensitive to prostaglandin $F_{2\alpha}$, 10^{-9} M and to oxytocin, 5×10^{-12} M and isotonically recorded dose-response curves were very steep.

Indomethacin action on uterine response to drugs

Figure 3 represents the action of indomethacin at a concentration of 5×10^{-6} M on the dose-response curves to the three agonists. Indomethacin markedly reduced the contractile response to oxytocin during proestrus in such a way that the response to the hormone in the presence of the inhibitor is practically shifted to the metoestrus level. Uterine response to oxytocin during metoestrus was not modified by indomethacin.

Response to angiotensin during metoestrus was also unmodified by indomethacin. On the other hand the inhibitor shifted the response to the polypeptide to the right during proestrus but did not completely abolish the proestrus increase in sensitivity as it did with oxytocin.

These results suggest that the observed increase in sensitivity to angiotensin and oxytocin in proestrus is due to stimulation of the synthesis of prostaglandins, a phenomenon which is apparently more marked with oxytocin. Prostaglandin $F_{2\alpha}$ responses during proestrus and dioestrus are not modified by indomethacin, which indicates that the contraction produced by exogenous prostaglandins probably does not involve endogenous prostaglandin synthesis.

Polyphloretin phosphate action on uterine response to drugs

The inhibitory action of PPP was explored during the different stages of the cycle. At the concentration of 10 μg/ml (Fig. 4), PPP shifted to the right the dose-response curves to oxytocin, angiotensin II and prostaglandin $F_{2\alpha}$ measured during dioestrus and proestrus, but did not modify oestrus and metoestrus responses to the three agonists. This result is similar to that obtained with indomethacin. On the other hand, at the concentration of 50 μg/ml (Fig. 5), PPP shifted all dose-response curves to the right, including those obtained in metoestrus. The effects obtained with the higher concentration of PPP are therefore different from those obtained with both the lower

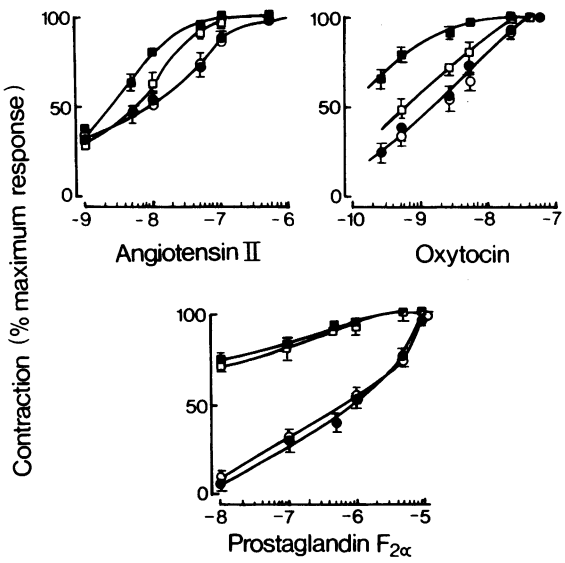

Fig. 3 Effect of indomethacin (5×10^{-6} M) on log dose-response curves to angiotensin II, oxytocin and prostaglandin $F_{2\alpha}$ in proestrus (■ □) and metoestrus (● ○). Responses in the presence (□ ○) or absence (■ ●) of the inhibitor expressed as percentages of the maximal induced contraction. Each point is the mean from 10-12 experiments; vertical bars show s.e. mean.

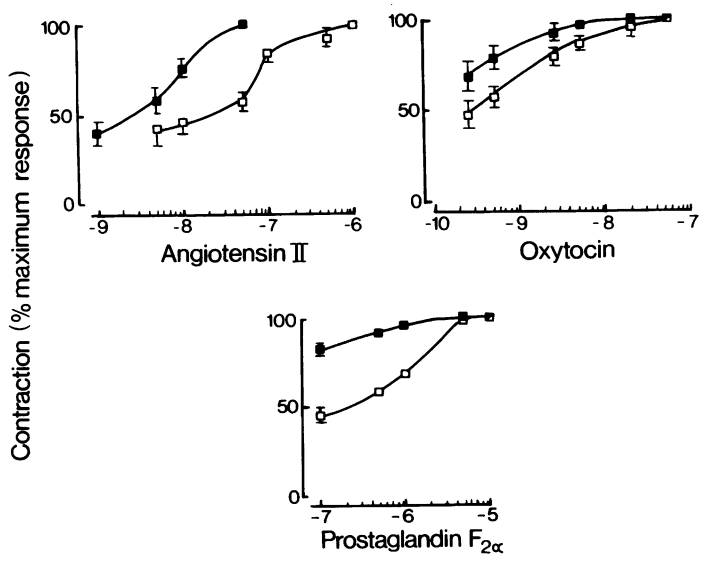

Fig. 4 Effect of polyphloretin phosphate (10 μg/ml) on the log dose-response curves to angiotensin II, oxytocin and prostaglandin $F_{2\alpha}$ in proestrus. Responses observed in presence (□) or absence (■) of the inhibitor expressed as percentages of the maximal induced contraction. Each point is the mean of 6 to 8 experiments; vertical bars show s.e. mean.

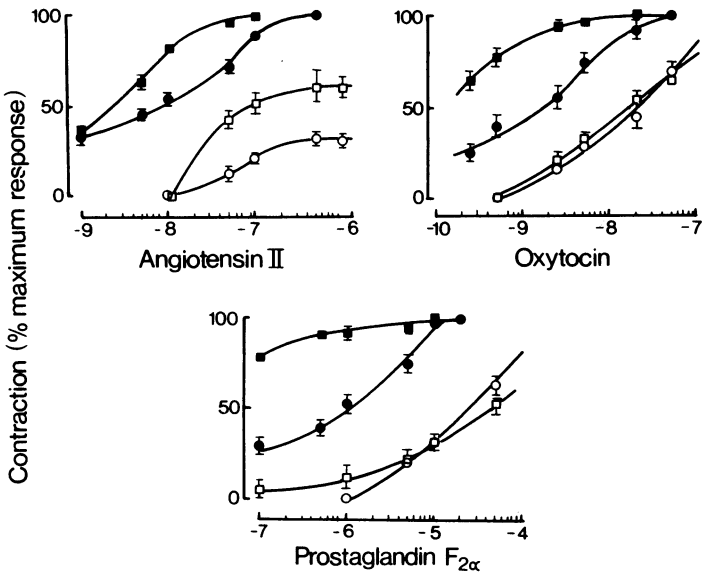

Fig. 5 Effect of polyphloretin phosphate (50 μg/ml) on the contractile responses to angiotensin II, oxytocin and prostaglandin $F_{2\alpha}$ observed in proestrus (■ □) and metoestrus (● ○). The contractions obtained in presence (□ ○) or absence (■ ●) of the inhibitor are expressed as percentages of the maximal response. Each point is the mean of eight experiments; vertical bars show s.e. mean.

dose of PPP and indomethacin. This result may be interpreted as due to release of prostaglandins during uterine contractions in all stages of the oestrous cycle or, alternatively, it may be that at the concentration of 50 μg/ml PPP non-specifically inhibits the direct spasmogenic action of all the active drugs tested.

Effect of prostaglandin $F_{2\alpha}$ on angiotensin spasmogenic activity

Dose-response curves to angiotensin II were determined after exposure of the uterus to 10^{-6} M prostaglandin $F_{2\alpha}$ left in the bath for 3 min and then washed out. After a subsequent 30 min incubation in Krebs solution, angiotensin-elicited contractions were again measured. This procedure resulted in a shift to the left of the metoestrus dose-response curve which became indistinguishable from the dioestrus and proestrus curves (10 experiments). In addition, the amplitude of the angiotensin contractile effect measured in dioestrus was increased by 30%.

Discussion

The variations of uterine smooth muscle response to three different agonists, observed throughout the oestrous cycle, emphasizes the functional importance of sexual steroid hormones in uterine contractility. Plasma oestrogen levels are extremely low and progesterone levels relatively high in oestrus and metoestrus. Conversely, dioestrus and more particularly proestrus are characterized by high oestrogen and rather low progesterone secretions (Hori, Ide & Miyake, 1968; Uchida, Kadowaki & Miyake, 1969). In agreement with many previous studies, this investigation demonstrates that oxytocin has a maximal contractile effect when natural oestrogen levels are high. This phenomenon is not specific for oxytocin but is also observed with angiotensin II and prostaglandin $F_{2\alpha}$. As far as we know the influence of sexual steroid hormones on angiotensin spasmogenic action has not been investigated. Conflicting results have been reported with prostaglandin $F_{2\alpha}$: Hawkins, Jessup & Ramwell (1968) found uterine desensitization to prostaglandin $F_{2\alpha}$ in castrated rats receiving oestrogen, and Sullivan (1966) did not observe any difference in prostaglandin $F_{2\alpha}$ responses in rat uteri sampled in dioestrus and after oestrogen administration. Conversely, Gans (1972) observed supersensitivity of the uterus to prostaglandin $F_{2\alpha}$ in dioestrus *in vivo*. These observations suggest that changes in uterine sensitivity brought about by sex hormones might be more directly related to

the oestrogen-progesterone ratio during the oestrous cycle, than to the absolute level of one of these two hormones.

The results of this study suggest that the increased uterine contractility observed during oestrogen dominance is related to endogenous prostaglandins: (i) spontaneous uterine contractions in high oestrogen periods of the oestrous cycle were abolished by indomethacin and PPP, and reproduced by exogenous prostaglandin $F_{2\alpha}$ in the metoestrus uterus. This suggests that the increased hyperactivity observed in proestrus and dioestrus is produced by an increased endogenous prostaglandin production. (ii) Increased response to oxytocin and angiotensin, observed during dioestrus and proestrus were reduced by indomethacin. (iii) Prostaglandin $F_{2\alpha}$ responses were also higher in the two high oestrogen situations. Since this increased sensitivity to prostaglandins is not affected by indomethacin, it may be concluded that increased uterine contractility is not only associated with an increased prostaglandin release or production but also with an increased sensitivity of the smooth muscle to prostaglandin $F_{2\alpha}$. (iv) Cycle-dependent increases in sensitivity to oxytocin and angiotensin were reduced by low concentrations of PPP. The interaction between angiotensin and oxytocin and endogenous prostaglandins demonstrated by our investigations agree with other results reported in the literature; angiotensin has been shown to cause prostaglandin release in other tissues (McGiff, Crowshaw, Terragno & Lonigro, 1970; Aiken & Vane, 1971; Douglas, Johnson, Marshall, Jaffe & Needleman, 1973).

Vane & Williams (1973) observed a decrease of the rat uterus contractile response to oxytocin with indomethacin and meclofenamate. Furthermore, there is an increase in prostaglandin concentration in endometrium at the end of pregnancy and when natural oestrogen levels are high. This is accompanied by an increased prostaglandin release in uterine venous blood (Challis, Harrison, Heap, Horton & Poyser, 1972; Thornburn & Currie, 1973) which can be suppressed by progesterone administration (Thornburn & Currie, 1973). The uterine prostaglandin released from the late pregnant uterus has been characterized as $F_{2\alpha}$ (Vane & Williams, 1973). In the pregnant rat uterus Aiken (1972) has found that the spontaneous contractility is related to prostaglandin release, and that this release is not a consequence of uterine contraction *per se*, since it was not affected by complete inhibition of the muscle contractions with papaverine.

Prostaglandin $F_{2\alpha}$ induces potentiation of both spontaneous uterine contractions and the response to angiotensin. This suggests that prostaglandins have an effect of long duration on some intermediary substance involved in the excitation-contraction process.

In summary, it is known that oestrogens induce uterine contractile responses to catecholamines (Diamond & Brady, 1966; Tothill, 1967; Paton, 1968), and it is likely that this effect is mediated by endogenous prostaglandins (Tothill *et al.*, 1971; Aiken, 1972; Vane & Williams, 1972). The present investigation has demonstrated that under oestrogen influence uterine contractility and sensitivity to angiotensin II and oxytocin are increased, this enhancement also being partially mediated by endogenous prostaglandins. Prostaglandins may be involved in the uterine contractile process by acting as intracellular modulators of uterine motility.

We wish to thank Dr J. Pike (The Upjohn Company, Kalamazoo, Michigan) for the generous supply of prostaglandins, Dr B. Högberg (Leo Laboratories, Hälsingborg) for PPP, and Mrs S. Hamon for typing the text. The work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (INSERM), the Centre National de la Recherche Scientifique (CNRS) and the Délégation Générale à la Recherche Scientifique et Technique (DGRST).

References

ABDEL-AZIZ, A. & BAKRY, N. (1972). The nature of the adrenoreceptors in the post-partum rat uterus. *Br. J. Pharmac.*, **45**, 504-509.

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. & VANE, J.R. (1971). Blockade of angiotensin-induced prostaglandin release from dog kidney by indomethacin. *The Pharmacologist*, **13**, 293.

BUTTERWORTH, K.R. & RANDALL, M.J. (1970). The effect of α- and β-adrenoceptor blocking agents on the responses of the rat uterus to catecholamines throughout the oestrus cycle. *Br. J. Pharmac.*, **40**, 160-161P.

CHALLIS, J.R.G., HARRISON, F.A., HEAP, R.B., HORTON, E.W. & POYSER, N. (1972). A possible rôle of oestrogens in the stimulation of prostaglandin $F_{2\alpha}$ output at the time of parturition in a sheep. *J. Reprod. Fert.*, **30**, 485-488.

DIAMOND, J. & BRADY, T.M. (1966). Effect of catecholamines on smooth muscle motility and phosphorylase activity. *J. Pharmac. exp. Ther.*, **152**, 202-211.

DOUGLAS, J.R., JR., JOHNSON, E.M., MARSHALL, G.R., JAFFE, B.M. & NEEDLEMAN, P. (1973).

Stimulation of splenic prostaglandin release by angiotension and specific inhibition by cysteine^8-AII. *Prostaglandins*, **3**, 67-74.

EAKINS, K.E., KARIM, S.M.M. & MILLER, J.D. (1970). Antagonism of some smooth muscle actions of prostaglandins by polyphloretin phosphate. *Br. J. Pharmac.*, **39**, 556-563.

GANS, P. (1972). Effects of prostaglandins and oxytocin on the uterine motility of the living uterus rat under various hormonal conditions. *Adv. Biosci.*, **9**, 783-788.

HAWKINS, R.A., JESSUP, R. & RAMWELL, P.W. (1968). Effect of ovarian hormones on response of the isolated rat uterus to prostaglandins. In *Prostaglandin Symposium of the Worcester Foundation for Experimental Biology*, ed. Ramwell, P.W. & Shaw, J.E., pp. 11-19. New York: Interscience.

HORI, T., IDE, M. & MIYAKE, T. (1968). Ovarian estrogen secretion during the estrus cycle and under influence of exogenous gonadotropins in rats. *Endocr. jap.*, **15**, 215-222.

McGIFF, J.C., CROWSHAW, K., TERRAGNO, N.A. & LONIGRO, A.J. (1970). Release of a prostaglandin-like substance into renal venous blood in response to angiotensin II. *Circulat. Res.*, **27**, suppl. 1, I121-130.

PATON, D.M. (1968). The contractile response of the isolated rat uterus to noradrenaline and 5-hydroxytryptamine. *Eur. J. Pharmac.*, **3**, 310-315.

SLIDER, E.M. & DOWNEY, H. (1950). Methods for the staining of leucocytes. In *McClung's Handbook of Microscopical Techniques*, ed. Jones, R.M., pp. 223-224. London etc.: Cassell.

SULLIVAN, T.J. (1966). Response of the mammalian uterus to prostaglandins under different hormonal conditions. *Br. J. Pharmac. Chemother.*, **26**, 678-685.

THORNBURN, D.G. & CURRIE, W.B. (1973). The physiology of the initiation of labour. In: *Les Prostaglandines*, ed. INSERM, pp. 259-277. Paris: INSERM.

TOTHILL, A. (1967). Investigation of adrenaline reversal in the rat uterus by the induction of resistance to isoprenaline. *Br. J. Pharmac. Chemother.*, **29**, 291-301.

TOTHILL, A., RATHBONE, L. & WILLMAN, E. (1971). Relation between prostaglandin E_2 and adrenaline reversal in the rat uterus. *Nature, New Biol.*, **233**, 56-57.

UCHIDA, K., KADOWAKI, M. & MIYAKE, T. (1969). Ovarian secretion of progesterone and 20α-hydroxypregn-4-en-3-one during rat estrus cycle in chronological relation to pituitary release of luteinizing hormone. *Endocr. jap.*, **16**, 227-237.

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. (1972). Prostaglandin production contributes to the contractions of the rat isolated uterus. *Br. J. Pharmac.*, **45**, 146P.

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.

(Revised April 24, 1974)