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THE ACTION OF BRADYKININ AND OXYTOCIN ON THE ISOLATED WHOLE UTERUS AND MYOMETRIUM OF THE RAT IN OESTRUS

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1 A technique is described for obtaining a myometrial preparation devoid of endometrium, from the uterus of the rat in oestrus.

2 Acetylcholine and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) produced concentration-effect curves with the same maximal tensions and slope on the whole uterus and myometrial preparations. Concentration-effect curves to bradykinin and oxytocin on the myometrial preparation were altered, resulting in a shift to the right and a decreased maximum response compared with those produced by the whole uterus.

3 Indomethacin produced greater antagonism of the responses of the whole uterus to bradykinin and oxytocin than to acetylcholine and $PGF_{2\alpha}$, whereas responses of the myometrium to all four agonists were similarly depressed.

4 Responses of the myometrial preparation to a range of concentrations of bradykinin and oxytocin were significantly enhanced by prior sensitization of the myometrium to $PGF_{2\alpha}$. This significant enhancing effect of $PGF_{2\alpha}$ was only seen with the threshold dose of acetylcholine.

5 It appears that the mechanism of action of bradykinin and oxytocin on the rat uterus involves both a direct action and an indirect action. The indirect action possibly involves release of prostaglandin(s) from the endometrium.

Introduction

Considerable support has developed for the view that prostaglandins released from the uterus, as a consequence of oxytocin-induced contractions, act synergistically with oxytocin to change myometrial activity (Brummer, 1971; Liggins, 1973). Vane & Williams (1973) have suggested that as well as inducing muscle contraction, oxytocin may stimulate the synthesis of prostaglandins which would then potentiate the contractions induced by oxytocin, perhaps by increasing the rate and spread of depolarization as suggested by Clegg, Hall & Pickles (1966). The isolated uterus of the rat is almost as sensitive to bradykinin as it is to oxytocin. It is possible that bradykinin exerts its uterine stimulant effect in a manner analagous to that postulated for oxytocin. In support of this hypothesis, bradykinin has been previously shown to release prostaglandins in a variety of tissues including rabbit kidney (Colina-Chourino, McGiff, Miller & Nasjletti, 1976) cat spleen (Ferreira, Moncada & Vane, 1973) and rat terminal ileum (Crocker & Willavoys, 1976).

In an attempt to provide more information on the problem, this study investigates the uterotonic action of bradykinin and compares it with that of oxytocin on the whole uterus and on a myometrial preparation devoid of endometrium, since it is the endometrium (at least in the pregnant rat) which appears to be the major source of prostaglandins (Williams, Sneddon & Harney, 1974).

Methods

Virgin Sprague-Dawley rats, 150–220 g, in natural oestrus were used. The stage of the oestrous cycle was determined by microscopic examination of the vaginal smear. Whole uterine horns were mounted in a 20 ml organ bath containing De Jalon's solution at 31°C bubbled with 95% O_2 and 5% CO_2. A resting tension of 0.5 g was applied to each tissue, and isometric contractions recorded on a pen recorder.

Myometrial preparations

To obtain myometrial preparations uteri were everted and a glass rod placed in the lumen. A thin layer

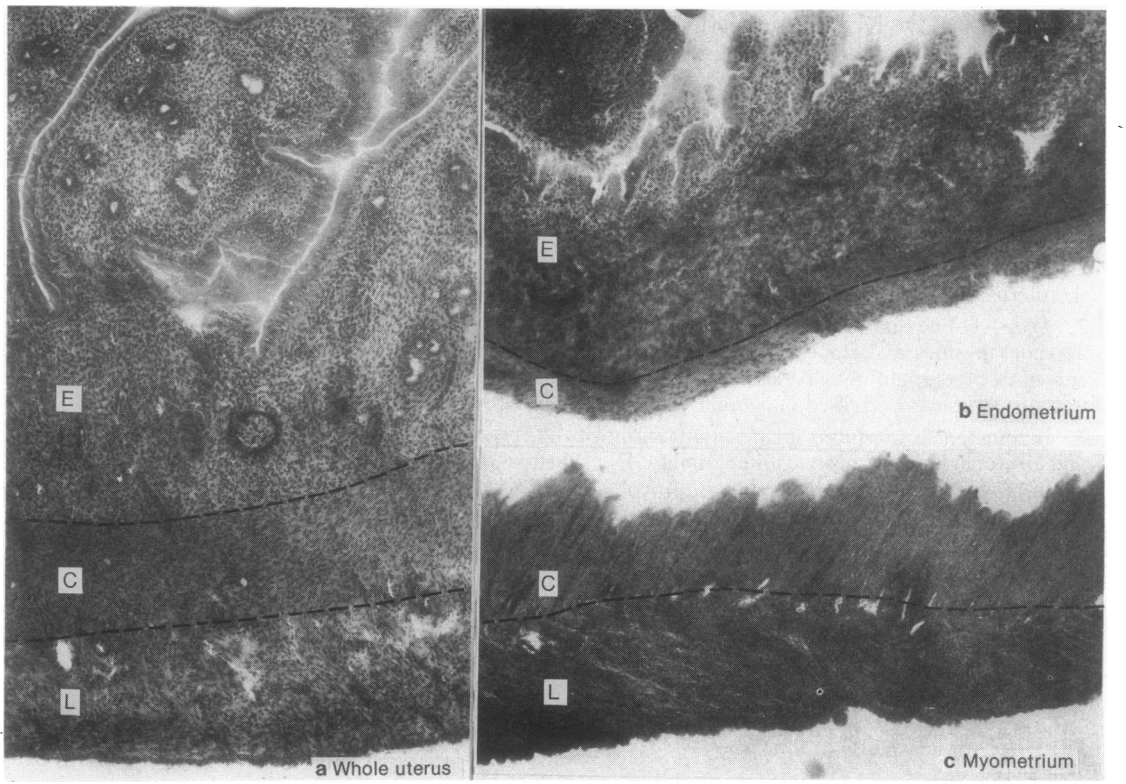

Figure 1 Histological sections of the rat uterus in oestrus before (a) and after separation of the endometrial tissue (b) from the myometrial tissue (c). E = Endometrium; C = Circular muscle layer; L = Longitudinal muscle layer. Magnification ($\times 100$).

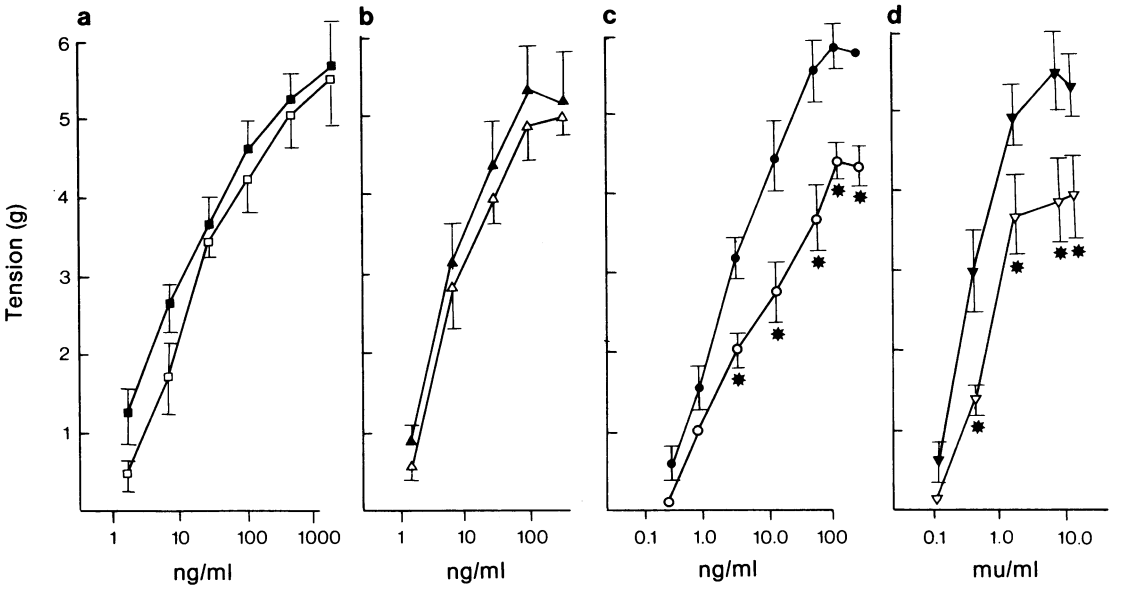

Figure 2 Concentration-effect curves for (a) acetylcholine (■), (b) prostaglandin $F_{2\alpha}$ (▲), (c) bradykinin (●) and (d) oxytocin (▼) on the whole uterus (closed symbols) and myometrium (open symbols). n = 5–6. *Significant difference in responses: $P < 0.05$.

of endometrium was removed from the cervical end of the uterine horn with fine forceps. The cervical end of the uterine horn was then anchored firmly and the burred end of the endometrial tissue picked up with forceps and the endometrium removed by gently pulling towards the ovarian end of the uterus. The endometrium could thus be removed intact leaving an intact myometrium on the glass rod.

The myometrial preparations were set up as for whole uteri. Drugs were added to the bath at 4 min intervals and left in contact with the preparation for 45 to 90 s. Tissues were incubated with antagonists for 45 min before doses of agonists were repeated.

Solutions and drugs

The composition of the De Jalon's solution (g/l) was: NaCl 9, KCl 0.42, $CaCl_2.6H_2O$ 0.472, $MgSO_4.7H_2O$ 0.38, glucose 1 and $NaHCO_3$ 0.5. Drugs used were as follows, doses being expressed in terms of salts: acetylcholine chloride (BDH), prostaglandin $F_{2\alpha}$-tromethamine (Upjohn), oxytocin, bradykinin triacetate (Sandoz), polyphloretin phosphate (Leo), indomethacin (Merck, Sharpe & Dohme).

Statistics

The statistical significance of the observed differences between mean values was tested by Student's t test.

Results

Histology

Figure 1a to c shows the histological appearance of the rat uterus in oestrus before and after stripping away the endometrial tissue. All sections demonstrated good separation of endometrial tissue from the myometrium. Cleavage was found to occur mainly within the circular muscle layer of the myometrium. As can be seen from Figure 1b, a thin strip of circular muscle is removed from the myometrium which remains attached to the endometrial tissue. The myometrial preparation was composed of a circular muscle layer which had a thin strip of muscle removed and an intact longitudinal muscle layer (Figure 1c).

Concentration-effect curves on whole uterus and myometrium

Concentration-effect curves were obtained with acetylcholine, prostaglandin $F_{2\alpha}$, bradykinin and oxytocin on the whole uterus and myometrial preparations (Figure 2). Acetylcholine and prostaglandin $F_{2\alpha}$ produced similar concentration-effect curves on the whole uterus and myometrial preparations with the same maximal tension being developed. The concentration-effect curves to bradykinin and oxytocin on

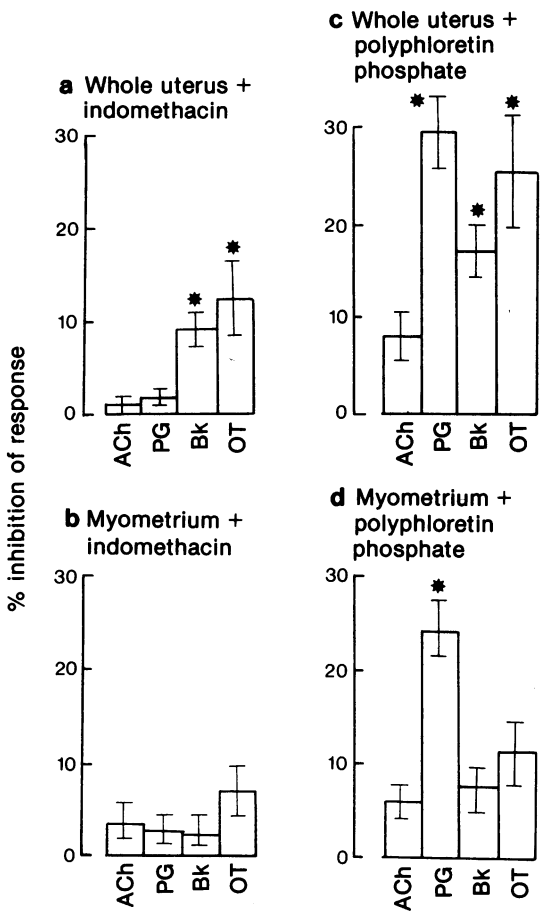

Figure 3 Mean percentage inhibition by indomethacin (5 μg/ml) and polyphloretin phosphate (50 μg/ml) of the response of the whole uterus and myometrium to concentrations of acetylcholine (ACh = 1 μg/ml), prostaglandin $F_{2\alpha}$ (PG = 100 ng/ml), bradykinin (Bk = 50 ng/ml) and oxytocin (OT = 10 mu/ml) which alone, produced just maximal contractions. *Inhibition of response which is significantly greater ($P < 0.05$) than that of acetylcholine. ($n = 4 - 5$).

the myometrial preparation were shifted to the right with significant reductions ($P < 0.05$) in the maximal tension produced, compared with those produced by the whole uterus.

Effects of indomethacin and polyphloretin phosphate on maximum responses to agonists

The doses of each agonist that produced a just maximal response on both the whole uterus and myometrial preparations were repeated in the presence and absence of indomethacin (5 μg/ml). The results are shown in Figure 3. On the whole uterus significant reductions ($P < 0.05$) in the response to oxytocin (12.2 ± 4.0%) and bradykinin (9.1 × 2.0%) were produced in the presence of indomethacin (Figure 3a) relative to acetylcholine (1.1 ± 0.9%). When this procedure was repeated with the myometrial preparation, only oxytocin showed an apparent reduction in response (7.8 ± 2.8%) in the presence of indomethacin (Figure 3b); however, this was not significantly different from that of acetylcholine (3.4 ± 3.0%).

The prostaglandin antagonist, polyphloretin phosphate, at a concentration of 50 μg/ml was shown to reduce not only the response of the whole uterus to maximal concentrations of prostaglandin $F_{2\alpha}$ (30.3 ± 5.3%) (Figure 3c) but also the response to oxytocin (26.5 ± 6.0%) and bradykinin (16.4 ± 3.8%). Acetylcholine was antagonized by 7.9 ± 2.2%. The antagonism of prostaglandin $F_{2\alpha}$, bradykinin and oxytocin by polyphloretin phosphate was significantly greater than that of acetylcholine ($P < 0.05$). With the myometrium, although responses to acetylcholine were apparently reduced by polyphloretin phosphate to the extent of 6.4 ± 2.8% only prostaglandin $F_{2\alpha}$ responses were significantly reduced (24.5 ± 2.7%, $P < 0.05$) relative to acetylcholine (Figure 3d). Responses to oxytocin were reduced by 12.8 ± 2.8% and bradykinin by 7.2 ± 2.4%, these being not significantly different from acetylcholine.

Prostaglandin enhancement of myometrial responses

Three concentrations of acetylcholine, bradykinin and oxytocin were chosen which covered the dose-response range to each agonist on the myometrial preparation. The selected concentrations of each agonist were repeated until constant responses were obtained. Prostaglandin $F_{2\alpha}$ (10 ng/ml) was added to the bath and left in contact with the tissue for 45 s after which the tissue was washed twice. The selected concentrations of each agonist were then repeated. The results are shown in Figure 4 for acetylcholine and bradykinin. All three concentrations of bradykinin chosen (Figure 4a, b, c) were enhanced by prior sensitization of the myometrium with prostaglandin $F_{2\alpha}$. In contrast to bradykinin only the low concentration of acetylcholine used (Figure 4d) was consistently significantly enhanced by prostaglandin $F_{2\alpha}$. As can be seen from Figure 4, the enhancing effect produced by prostaglandin $F_{2\alpha}$ lasted for several dose cycles, with a gradual return to normal responses. Oxytocin was also enhanced at all dose levels used by prostaglandin $F_{2\alpha}$. The first response to each agonist after sensitization of the myometrium with prostaglandin $F_{2\alpha}$ was compared with the last response prior to sensitization and the mean results for all three agonists are shown in Figure 5.

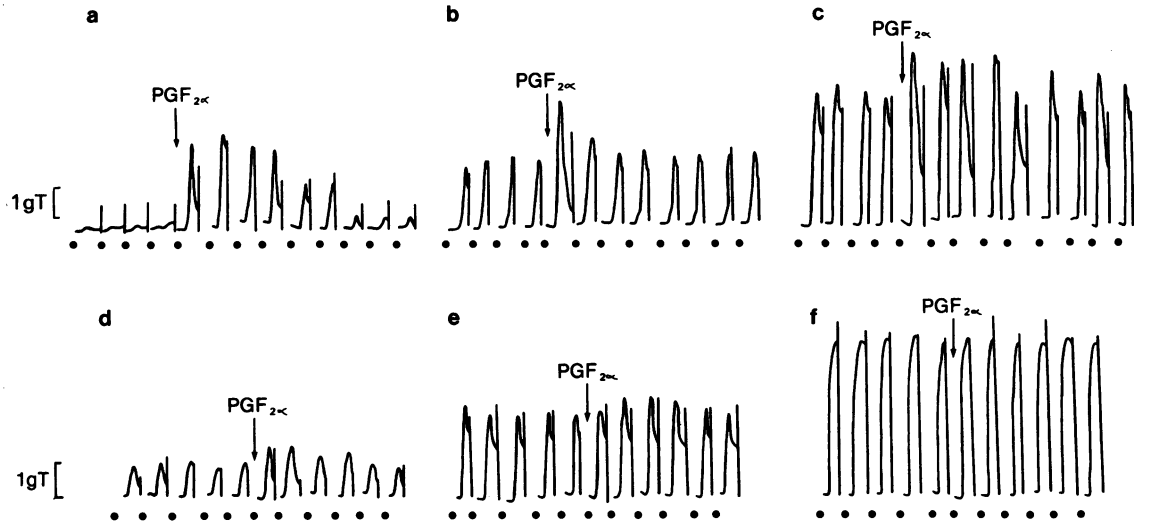

Figure 4 Responses of the myometrial preparation to selected concentrations of bradykinin (a) 0.2 ng/ml; (b) 10 ng/ml and (c) 50 ng/ml and of acetylcholine: (d) 2 ng/ml; (e) 24 ng/ml and (f) 1000 ng/ml before and after sensitization of the preparation with prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$ 10 ng/ml).

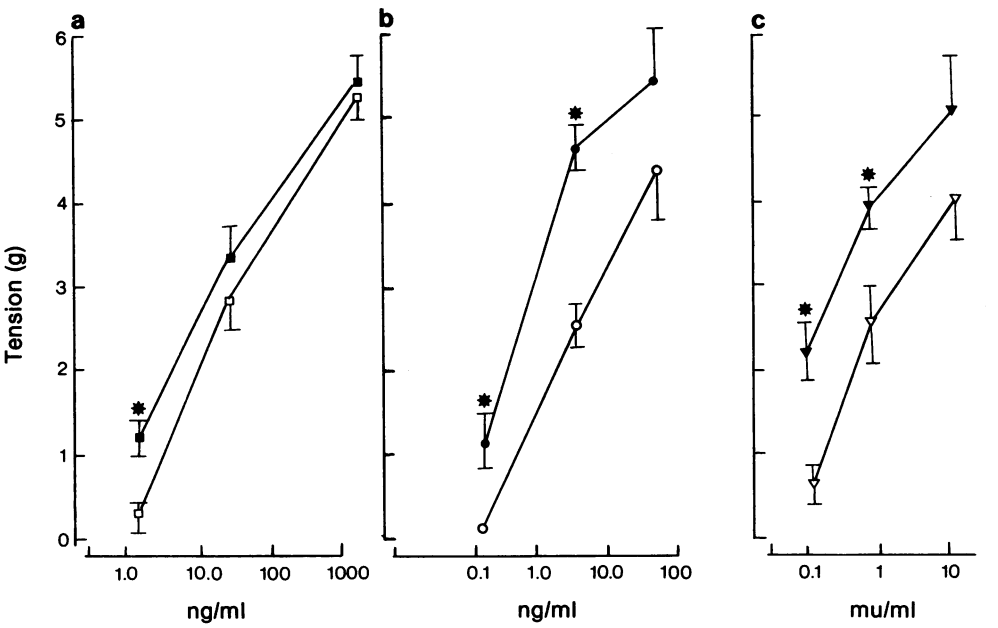

Figure 5 Concentration-effect curves to (a) acetylcholine (□), (b) bradykinin (○) and (c) oxytocin (▽) before (open symbols) and after (closed symbols) sensitization of the myometrium to prostaglandin $F_{2\alpha}$ (10 ng/ml). n = 5–6. *Significant potentiation, $P < 0.05$.

Discussion

Since Karim & Devlin (1967) and Karim & Sharma (1971) suggested that prostaglandins may have a physiological oxytocic action, much evidence has been produced to suggest that they may play a key role in myometrial activation (Csapo, 1973). This includes control of the spontaneous activity of the uterus and its electrical excitability, and reactivity to various stimulants (Csapo & Csapo, 1974). Vane & Williams (1973) found that the contractions of the isolated uterus of the non-pregnant rat, elicited by oxytocin were antagonized by indomethacin at a concentration known to inhibit prostaglandin synthesis, whereas responses evoked by prostaglandin $F_{2\alpha}$ and acetylcholine were not. Two possible explanations were suggested by Vane & Williams (1973) for the antagonism produced by indomethacin on the oxytocin response. First, there may be a continuous prostaglandin synthesis which sensitizes the uterus to oxytocin. It is well established that prostaglandins potentiate responses to oxytocin on the rat isolated uterus and on strips of human myometrium (Pickles, Hall, Clegg & Sullivan, 1966; Brummer, 1972). Second, oxytocin (but not acetylcholine or prostaglandin $F_{2\alpha}$) as well as inducing muscle contraction, may stimulate the synthesis of prostaglandins which would then potentiate the contractions induced by oxytocin, perhaps by increasing the rate and spread of depolarization, as suggested by Clegg *et al.* (1966). The rat isolated uterus is almost as sensitive to bradykinin as it is to oxytocin. It is possible that bradykinin activates the uterus in a similar fashion to oxytocin, as bradykinin has been shown to release prostaglandins in a variety of tissues (see Introduction).

The endometrium appears to be the major source of prostaglandins, at least in the pregnant rat uterus (Williams *et al.*, 1974; Downing & Williams, 1977). Therefore the use of a myometrial preparation devoid of endometrial tissue provided a mechanism by which the action of bradykinin and oxytocin could be further investigated. As the stripped preparation, free from endometrial tissue was still responsive to bradykinin and oxytocin, this may indicate a direct action of these two peptides on the myometrium. However, their effects on the whole uterus may also involve a 'prostaglandin component' as indicated by the difference in the response of the whole uterus and myometrium to these two peptides, and the reduction in response of the whole uterus to these peptides after indomethacin and polyphloretin phosphate. These results confirm previous studies that have demonstrated an inhibitory effect of indomethacin on the response of the whole uterus to oxytocin (Vane & Williams, 1973; Baudouin-Legros, Mayer & Worcel, 1974) and bradykinin (Sorrentino, Capasso & Di Rosa, 1972; Barbaré Park & Regoli, 1975). Similarly, polyphloretin phosphate has been shown previously to antagonize partially the response of the whole uterus to oxytocin (Barbaré *et al.*, 1975).

The amount of prostaglandin that would have to be released endogenously to produce potentiation of agonist action is difficult to estimate. It has been shown in this study that responses produced by bradykinin and oxytocin throughout the concentration range studied can be significantly enhanced by prior sensitization of the myometrium with small concentrations of prostaglandin $F_{2\alpha}$. In contrast to bradykinin and oxytocin, the effect of prior sensitization of the myometrium with prostaglandin $F_{2\alpha}$ resulted in enhanced responses only with threshold concentrations of acetylcholine.

However, it should be noted that although bradykinin and oxytocin appear to have a distinct direct action on the myometrium, it was not possible to produce maximal responses equivalent to those seen with the whole uterus.

In the pregnant rat uterus Aiken (1972) found that the spontaneous contractility is related to prostaglandin release and that this release is not a consequence of uterine contractions *per se*, since its production was not affected when contractions of the muscle were completely inhibited by papaverine.

This may suggest that bradykinin and oxytocin possess two distinct actions, a uterotonic action and a prostaglandin-releasing action, both being separate functions produced by action on receptors located at different sites. This is in contrast to acetylcholine which appears to have a predominantly direct uterotonic action.

Roberts & McCracken (1976), investigating the oxytocic action of oxytocin in sheep *in vivo*, concluded that while increased contractile activity is not a '*sine qua non*' for oxytocin-induced synthesis of prostaglandin $F_{2\alpha}$, neither is increased synthesis of prostaglandin $F_{2\alpha}$ an essential intermediate step in the activation of the myometrium by oxytocin. More recently Chan (1977) using rat isolated uteri has suggested that the myometrial receptors for oxytocin and those for prostaglandin are functionally separate and distinct and that the uterotonic action of oxytocin is independent of prostaglandin-participation. However, although the involvement of prostaglandin in the uterotonic action of oxytocin does not appear to be obligatory, it seems to be dependent upon the hormonal environment as demonstrated by Laudanski, Akerlund & Batra (1977) using rabbit uterus *in vivo*.

The capacity of the uterus to synthesize prostaglandins increases towards term in the rat (Vane & Williams, 1973; Williams *et al.*, 1974; Harney, Sneddon & Williams, 1974; Chan, 1977) and this may be related to the rapidly changing hormonal environment that occurs over the last few days of gestation in the rat. Mobilization of the kinin system is thought

to occur in late pregnancy and parturition in the rat (McCormick & Senior, 1974; Whalley & Riley, 1978) and humans (Martinez, De Carvalho & Diniz, 1962). Parturition can not only be delayed or prolonged in rats by use of inhibitors of prostaglandin synthesis such as aspirin and indomethacin (Aiken, 1972; Chester, Dukes, Slater & Walpole, 1972), but also by prevention of activation of the kinin system by use of kallikrein inhibitors (Senior & Whalley, 1976; Whalley & Riley, 1978). The increased capacity of the uterus to synthesize prostaglandins at term, and mobilization of the kinin system associated with the release of oxytocin from the pituitary could be related, resulting in activation of the uterus at term via direct and indirect actions of bradykinin and oxytocin, and spasmogenic action of these peptides being potentiated by the presence of increasing concentrations of prostaglandins produced by the uterus.

In conclusion, these results suggest that the action of bradykinin and oxytocin on the whole uterus of the rat in oestrus involves not only a significant direct action on the myometrium but also an indirect action via release of prostaglandin(s) from the endometrium.

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