# Reduction in vivo of uterine responses to bradykinin by indomethacin in the rat

E. T. WHALLEY, *Department of Pharmacology, Materia Medica & Therapeutics, Stopford Building, University of Manchester, Oxford Road, Manchester M13 9PT U.K.*

The action of bradykinin on the in vivo uterus is unclear and it has been shown to have no effect in several species, including humans, and to be weakly excitatory in others (Berde & Saameli 1961; Sturmer & Berde 1963; Bissett & Lewis 1962; Fregnan & Glasser 1964; Bissett et al 1965). However, Sicuteri et al (1962) produced labour-like uterine contractions in women at term after intravenous administration of bradykinin. The in vitro rat uterus has been shown to be very sensitive to bradykinin (Konzette & Sturmer 1960; Sturmer & Berde 1963; Vane 1964), and this action appears to involve a significant direct effect on the myometrium and an indirect action possibly mediated via release of a prostaglandin(s) from the endometrium (Whalley 1978; Whalley & Raynes 1979).

This study investigates the action of bradykinin and cellulose sulphate, a compound known to promote intrinsic formation of kinins (Rothschild 1968) on the in vivo uterus of the anaesthetized non-pregnant rat and the possible involvement of prostaglandins in the oxytocic action of bradykinin.

Virgin female Sprague-Dawley rats (180–200 g) in oestrus were used, the state of the oestrus cycle being determined by microscopic examination of the morning vaginal smear. Selected rats were anaesthetized with pentobarbitone sodium (45 mg $kg^{-1}$ i.p.) and prepared with an intra-uterine microballoon, inserted into the ovarian end of the uterine horn, for recording pressure changes within the uterus. A cannula was placed in the jugular vein for the intravenous injection of drugs. Intra-uterine pressure was recorded continuously using a Bell & Howell pressure transducer connected to a Grass polygraph. The effect of bolus intravenous injections of bradykinin were investigated before and after treating the animal with either saline, 0·9% w/v i.p., the cyclo-oxygenase inhibitor indomethacin, 2 mg $kg^{-1}$ i.p., or the kallikrein inhibitor soya bean trypsin inhibitor (S.B.T.I.), 20 mg $kg^{-1}$ i.p. Separate groups of rats were also pretreated with saline, indomethacin or S.B.T.I. at the above doses and the effect of a single intravenous injection of cellulose sulphate (1·0 mg $kg^{-1}$). The effect of $PGF_{2\alpha}$ on the uterus was studied before and after indomethacin to determine the selectivity of the cyclo-oxygenase inhibitor.

Drugs used were: bradykinin, Sandoz Ltd; indomethacin (Merck, Sharpe & Dohme) soya bean trypsin inhibitor (Sigma) cellulose sulphate was prepared by the method of Astrup et al (1944), prostaglandin $F_{2\alpha}$ (Upjohn).

The uterus was quiescent in most preparations, see Fig. 1, which also shows changes in intra-uterine pressure to increasing bolus intravenous doses of bradykinin. The responses to bradykinin were dose-related, the form of which was a smooth single peaked contraction. The responses of the uterus to an intravenous injection of cellulose sulphate was different to that of bradykinin, and after a rapid rise in intra-uterine pressure (with a maximum pressure, equivalent to that produced by 5$\mu$g bradykinin), a second cyclic phase followed, which returned to the quiescent state after 8–10 min.

The effect of the enzyme inhibitors used on the response of the uterus to bradykinin and cellulose sulphate are shown in Table 1. Indomethacin treatment reduced responses of the uterus to bradykinin over the whole dose range studied, these being significant ($P < 0.05$) at the higher doses. S.B.T.I. did not significantly affect the responses to bradykinin. The uterine response to cellulose sulphate was significantly reduced ($P < 0.05$) by indomethacin and completely abolished by S.B.T.I. Indomethacin had no effect on $PGF_{2\alpha}$-induced contractions.

These results demonstrate that bradykinin stimulates the in vivo uterus of the anaesthetized non-pregnant rat. Several factors may influence the oxytocic action of bradykinin given by the intravenous route and these include a short half-life due to rapid pulmonary clearance of the peptide (Ferreira & Vane 1967) and release of catecholamines from the adrenal medulla by bradykinin (Feldberg & Lewis 1964) and by the anaesthetic (Spriggs 1965) resulting in inhibitory uterine tone as a consequence of $\beta$-adrenoceptor activation. Any one or several of those factors may account for the considerable inconsistencies in the observations on the effect of bradykinin on the uterus in vivo (see Introduction). In this study however the responses of the uterus to bradykinin was clearly dose related, the form of the contraction being similar to that described by Fregnan & Glasser (1964) and Bissett et al (1965). A situation where bradykinin is released in excess of physiological quantities is provided by the administration of cellulose sulphate which promotes the intrinsic formation of kinins (Rothschild 1968). The dose of cellulose sulphate used in this study (1 mg $kg^{-1}$) has been shown result in 30–40% depletion of kininogen in the pregnant rat (McCormick et al 1974). Production of free kinin in the immediate environment of the uterus would result in a greater activation of the uterus. This was the case, the response to cellulose sulphate being much more prolonged and cyclic in form compared with the single peak effect as a result of exogenous administration of bradykinin. The myometrial response to cellulose sulphate was effectively blocked by the kallikrein inhibitor S.B.T.I., thus indicating that kinin generation involves activation of plasma kallikrein. In contrast, the responses to

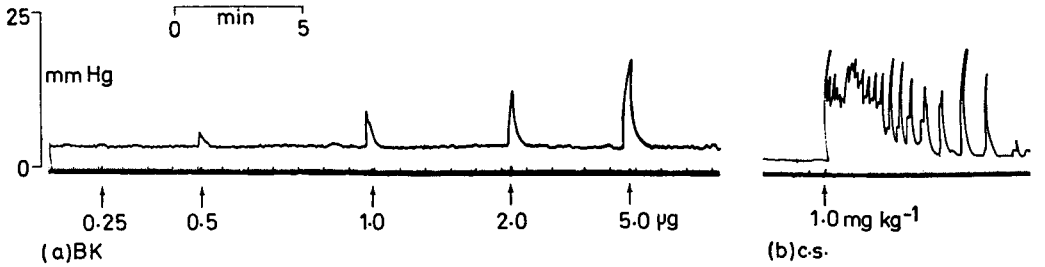


FIG. 1. Trace showing the effect of (a) bradykinin (BK 0·25–5·0 ug i.v.) and (b) cellulose sulphate (c.s. 1 mg $kg^{-1}$ i.v.), on uterine activity in the non-pregnant anaesthetized rat in vivo.

bradykinin were not significantly affected by treatment with S.B.T.I. The prostaglandin synthetase inhibitor indomethacin significantly reduced responses to the higher doses of bradykinin and to cellulose sulphate but did not completely abolish them. This suggests that a prostaglandin(s) may be involved in the uterine action of bradykinin in vivo and since significant inhibition was only seen against higher doses of bradykinin and cellulose sulphate, bradykinin may also have a direct action on the myometrium as appears to be the case with the in vitro rat uterus (Whalley 1978; Whalley & Raynes 1979). Activation of kinin

Table 1. The effect of indomethacin (2 mg $kg^{-1}$ i.p.) and S.B.T.I. (20 mg $kg^{-1}$ i.p.) on the maximum increase in uterine pressure produced by bradykinin (BK) and cellulose sulphate (c.s.). n = 4–6/group $*P < 0.05$ (Student's $t$-test)

| BK µg: | Saline control | indomethacin | S.B.T.I. |
|---|---|---|---|
| 0·25 | 4·84 ± 1·2 | 3·56 ± 0·56 | 3·83 ± 0·31 |
| 0·5 | 5·78 ± 1·86 | 2·90 ± 0·9 | 3·91 ± 0·34 |
| 1·0 | 9·06 ± 1·5 | 3·8 ± 1·04* | 7·31 ± 1·58 |
| 2·0 | 10·98 ± 2·02 | 4·42 ± 1·2* | 8·13 ± 0·58 |
| 5·0 | 15·84 ± 1·36 | 7·98 ± 1·54* | 13·64 ± 1·66 |
| c.s. mg $kg^{-1}$ | | | |
| 1·0 | 14·33 ± 0·92 | 6·85 ± 0·85* | 0 |

is thought to occur in late pregnancy and parturition in the rat (McCormick & Senior 1974) and kinins may be involved in uterine control mechanisms during parturition directly or indirectly since the kallikrein inhibitors S.B.T.I. and aprotinin have been shown to prolong pregnancy and parturition in rats (Senior & Whalley 1976; Whalley & Riley 1978; Whalley & Riley 1979).

In conclusion the action of bradykinin on the in vivo non-pregnant rat uterus appears to involve a prostaglandin component. Cellulose sulphate may be a useful tool in investigating the action of endogenously released kinin on uterine control mechanisms.

I wish to thank Sandoz Limited for the gift of synthetic bradykinin and Dr J. Pike, Upjohn Co. Ltd for $PGF_{2\alpha}$.

December 14, 1978

## REFERENCES

Astrup, T., Galsmar, B., Volkert, M. (1944) Acta Physiol. Scand. 8: 215–222.

Berde, B., Saameli, K. (1961) Nature (London) 191: 83

Bissett, G. W., Haldar, J., Lewin, J. E. (1965) Mem. Soc. Endocrinol. 14: 185–198

Bissett, G. W., Lewis, G. P. (1962) Br. J. Pharmacol. Chemother. 19: 168–182

Feldberg, W., Lewis, G. P. (1964) J. Physiol. (Lond.) 171: 98–108

Ferreira, S. H., Vane, J. R. (1967) Br. J. Pharmacol. Chemother. 30: 417–424

Fregnan, G. B., Glasser, A. H. (1964) J. Pharm. Pharmacol. 16: 744–750

Konzette, M., Sturmer, E. (1960) Br. J. Pharmacol. Chemother. 15: 544

McCormick, J. T., Senior, J., Whalley, E. T. (1974) Br. J. Pharmacol. 52: 533–537

McCormick, J. T., Senior, J. (1974) Ibid. 50: 237–241

Rothschild, A. M. (1968) Br. J. Pharmacol. Chemother. 33: 501–512

Senior, J., Whalley, E. T. (1976) J. Reprod. Fert. 47: 319–323

Sicuteri, F., Centaro, A., Massi, S. B., Periti, P. (1962) Boll. Soc. Ital. Biol. Sper. 38: 66–70

Spriggs, T. L. B. (1965) Br. J. Pharmacol. 24: 752–758

Sturmer, E., Berde, B. (1963) J. Pharmacol. Exp. Ther. 140: 349–355

Vane, J. R. (1964) Br. J. Pharmacol. Chemother. 23: 360–373

Whalley, E. T. (1978) Br. J. Pharmacol. 64: 21–28

Whalley, E. T., Riley, A. J. (1978) J. Endocrinol. 77: 20–21P

Whalley, E. T., Riley, A. J. (1979) J. Reprod. Fert. 55: 377–384

Whalley, E. T., Raynes, G. (1979) J. Endocrinol. 81: 134P–135P