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Indomethacin in low concentration potentiates the actions of some spasmogens on the isolated oestrous rat uterus

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In a number of smooth muscle tissues, the spasmogenic activity of bradykinin has been reported to involve the release of prostaglandins (Piper & Vane, 1969; Palmer, Piper & Vane, 1973; Crocker & Willavoys, 1976). The tissues of the isolated rat uterus synthesize prostaglandins (Williams, 1973) and spontaneous contractions of the rat uterus have been shown to be mediated by their release (Vane & Williams), 1973.

While testing the effectiveness of indomethacin, a prostaglandin synthetase inhibitor (Vane, 1971), in suppressing spontaneous contractions, we noted that indomethacin has another unexpected action on the isolated oestrous rat uterus.

Virgin Wistar rats 180–220 g, were brought to artificial oestrus, 20–22 h after a single subcutaneous injection of stilboestrol (20 μg per 100 g body weight) in 40% (v/v) aqueous ethanol. The animals were killed and exsanguinated. The uterine horns were excised and a length of 1–2 cm was suspended in 1·5 ml de Jalon solution (composition, g litre^{-1}: NaCl, 9; KCl, 0·42; $CaCl_2$, 0·06; $NaHCO_3$, 0·5; glucose, 0·5) at 35°, gassed with O_2/CO_2 (95:5%) and containing atropine sulphate (1 μg ml^{-1}).

Contractions were detected using an isotonic transducer with a load on the tissue of 0·5 g. Tissues were allowed to wash in de Jalon solution for 1 h before testing, to permit full relaxation. A 5 min dose cycle was used. Spasmogens remained in contact with the tissues for 70 s. The concentrations of indomethacin remained in contact with the tissue for the whole of the cycle. Every concentration of bradykinin and indomethacin was tested at least six times. Possible pH changes due to the various concentrations of indomethacin were monitored using a pH meter. Statistical significance of differences was determined using Student's *t*-test for paired differences.

Indomethacin in low concentration was found to potentiate contractions of the rat uterus produced by bradykinin (Fig. 1). Maximum potentiation was reached between 50 s and 10 min after contact of the tissue with indomethacin. The contraction size returned to the control value within 1 h of washing out the indomethacin. Parallel dose-response curves to bradykinin obtained in the presence of increasing concentrations of indomethacin, 0–0·56 μM (0–200 μg litre^{-1}) are shown in Fig. 2. A concentration of indomethacin as low as 0·14 μM produced detectable potentiation of the uterine contractions. The potentiation appears to be non-specific, since initial studies show that both acetylcholine and 5-HT are similarly potentiated by 0·56 μM indomethacin ($P<0·01$, n = 6). Indomethacin itself caused no change in the resting length of the tissue nor did it at any of its concentrations produce detectable alterations of pH.

Indomethacin (2·8 μM) has recently been reported to inhibit contractions of rat intestine produced by brady-

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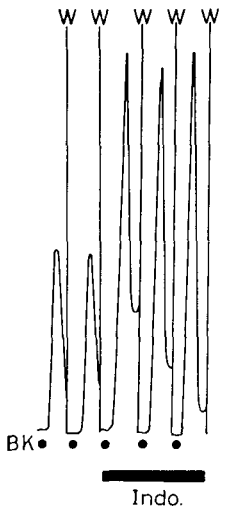

FIG. 1. Potentiation by indomethacin (100 ng ml^{-1}) (Indo) of bradykinin-induced contractions of isolated oestrous rat uterus. A 5 min cycle was used, with 70 s contact. The trace was stopped between doses. Closed circles indicate addition of 1 ng bradykinin (BK) to the bath. Wash out contractions shown by W.

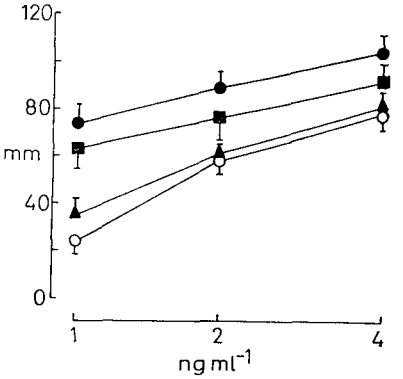

FIG. 2. Dose-response curves (semi-log scale) plotted for contractions of the oestrous rat isolated uterus in the presence of different concentrations of indomethacin. Mean ± s.e.m. n = 6. ●—5·6 × 10^{-7}M, ■—2·8 × 10^{-7}M, ▲—1·4 × 10^{-7}M, ○—Control. Ordinate—Response (4×)(mm). Abscissa—Bradykinin (ng ml^{-1}).

kinin (Crocker & Willavoys, 1976). On the isolated rat uterus, we found one tenth of this concentration to potentiate the spasmogenic action of bradykinin. This contrasts with the drug's inhibitory action on the contractions of rat uterus produced by oxytocin (Vane & Williams, 1973). Vane & Williams (1973) mentioned an inconsistent potentiation of the spasmogenic action of acetylcholine produced by 2·8 μM indomethacin on the rat isolated uterus. We found the much lower concentrations of 0·56 μM indomethacin always produced potentiation of the acetylcholine response.

It seems unlikely that the potentiation of spasmogenic activity produced by such low concentrations involves the inhibition of prostaglandin synthesis. However, the possibility that indomethacin could be concentrated in the uterine tissue cannot, at present, be ruled out. It has recently been shown that incubating intestinal smooth muscle with low concentrations of indomethacin, similar to those used in the present study (0·5 μM), lowers the tissue calcium concentration (Northover, 1972). The effect of a chelating agent on tissue calcium is likely to be exaggerated by the fact that de Jalon solution contains only 0·5 μM calcium, which is a fifth of the concentration in Krebs' solution and a sixth of the calcium in rat serum. Daniel & Irwin (1965) have concluded that the initial effect of calcium chelating agents on the rat uterus is to make the myometrial cell membrane unstable. The possibility that indomethacin may potentiate contractions of the rat uterus due to bradykinin by such a mechanism is now being evaluated.

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