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Prostaglandin synthesis inhibitors: effect on angiotensin II- and oxytocin-induced contractions in rat uterine smooth muscle

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1 Eicosatetraynoic acid, the acetylene analogue of arachidonic acid, which inhibits both the cyclo-oxygenase and lipoxygenase pathways, reduced the contractile response of rat uterine smooth muscle to either angiotensin II or oxytocin.

2 Indomethacin, an inhibitor of cyclo-oxygenase, did not reduce the response to angiotensin II but did abolish the contractile response to low doses of oxytocin.

3 Nordihydroguaiaretic acid, a lipoxygenase inhibitor, totally abolished the uterine response to either oxytocin or angiotensin II.

4 The contractile response to carbachol, a cholinoceptor agonist, was unaffected by pretreatment with any of the cyclo-oxygenase or lipoxygenase inhibitors.

5 From these findings, it can be implied that some product of the arachidonate lipoxygenase pathway augments peptide-induced contractions of the rat uterus.

Introduction

Prostaglandin synthesis inhibitors have been shown to attenuate the activity of angiotensin II (Ang II) in a number of tissues (Aiken & Vane, 1971; Chong & Downing, 1973; Gimbrone & Alexander, 1974; Goodfriend & Simpson, 1981). Both Ang II and oxytocin induce a contractile response in rat uterus; it is apparent that this action is in some way dependent on prostaglandin synthesis (Vane & Williams, 1973; Baudouin-Legros, Meyer & Worcel, 1974; Ghodgaonkar & King, 1979; Dubin & Ghodgaonkar, 1980). In the above studies the anti-inflammatory drug, indomethacin, was used as the cyclo-oxygenase inhibitor of prostaglandin synthesis (Flower, 1974).

In the present study, there is a comparison between the effects of indomethacin, the effects of eicosatetraynoic acid (ETYA), which inhibits both the cyclo-oxygenase and lipoxygenase pathways (Hamberg, Svensson & Samuelsson, 1974; Hamberg & Samuelsson, 1974), and the effects of nordihydroguaiaretic acid (NDHGA), a lipoxygenase inhibitor (Hamberg, 1976) on the contractile response of rat uterus to Ang II, oxytocin and carbachol.

Methods

Female Sprague Dawley rats weighing 180–225 g were pretreated 16–18 h before experimentation, intraperitoneally, with 15 mg/kg stilboestrol in cotton seed oil (Bergman, 1968). Before the rats were killed, vaginal smears were taken to establish oestrus. The rats were killed by cervical dislocation and the uterine horns removed. A segment, approximately 1.5 cm in length, was suspended in a 5 ml Metroware organ bath containing de Jalon's solution, and aerated with 95% O_2, 5% CO_2 at a constant temperature of 32°C. The composition of de Jalon's solution was as follows (mM): NaCl 153, KCl 5.6, $CaCl_2$ 0.54, $NaHCO_3$ 5.95 and glucose 2.77. The uterine horns were attached to a Grass force displacement transducer (FT 83C) under 2 g of tension and allowed to equilibrate for 30 min. Isometric contractions were measured. The transducer was connected through a type 9853A coupler to a Beckman R612 recorder.

Ang II and oxytocin dose ranges were calculated on the basis of the minimum concentration required for contraction (Bisset & Lewis, 1962). A dose-response curve was obtained with doses of Ang II, 8

and 64 ng/ml, recording isometric contractions in g. Oxytocin dose-response curves were constructed using dosages of 4 and 20 mu/ml.

Following the initial dose-response curve, for each polypeptide separately, the uteri were continuously preincubated with de Jalon's solution containing various doses of indomethacin, ETYA or NDHGA at various times. Uteri were pre-incubated with indomethacin (2×10^{-6}, 2×10^{-5} M) for 5, 15 and 30 min; doses of ETYA (4×10^{-5} M) and NDHGA (10^{-5} M) were given for the same time periods. A single dose of carbachol (20 μg/ml) was tested before and during the incubation with these inhibitors at the same time periods.

Indomethacin, ETYA and NDHGA were suspended in 2 ml absolute ethanol stock solution and then diluted with de Jalon's solution to the appropriate concentrations.

The final concentration of ethanol in the bath did not exceed 0.1%. During inhibition of arachidonic acid conversion, dose-response curves to Ang II or oxytocin were constructed. A single dose of carbachol (20 μg/ml) was also tested. After the final peptide dose, uteri were washed with fresh de Jalon's solution and 5 min later the tissue's responsiveness to the peptide or carbachol was again tested to ascertain the recovery of the tissue.

Statistical significance of differences between control and experimental responses were determined by Student's *t* test for paired differences. Drugs were supplied by the following: 5, 8, 11, 14-eicosatetraynoic acid by W.E. Scott, Hoffman La Roche, Inc.; angiotensin II, by Dr M.C. Khosla, Cleveland Clinic Foundation; indomethacin by Merck, Sharpe and Dohme; nordihydroguaiaretic acid by Aldrich Biochemicals; oxytocin by Sigma Chemical Corporation. Doses of the prostaglandin synthesis inhibitors are similar to those reported in the literature: indomethacin (2×10^{-6}, 2×10^{-5} M) (Vane & Williams, 1973; Baudouin-Legros *et al.*, 1974; Brink, Duncan & Douglas, 1981), ETYA (4×10^{-5} M) and NDHGA (10^{-5} M) (Hamberg, 1976).

Results

Ang II-induced contractions of oestrogen-treated rat uteri were not abolished by pretreatment with indomethacin (Figure 1). The anti-inflammatory drug was without effect at 5, 15 or 30 min pretreatment. After washing with de Jalon's solution (5 min) the tissue's response to Ang II was the same as control.

The contractile response to Ang II was abolished by ETYA (Figure 1). At 5, 15 or 30 min pretreatment with ETYA the response to Ang II was significantly reduced ($P < 0.001$). After washout of ETYA, the response to Ang II returned.

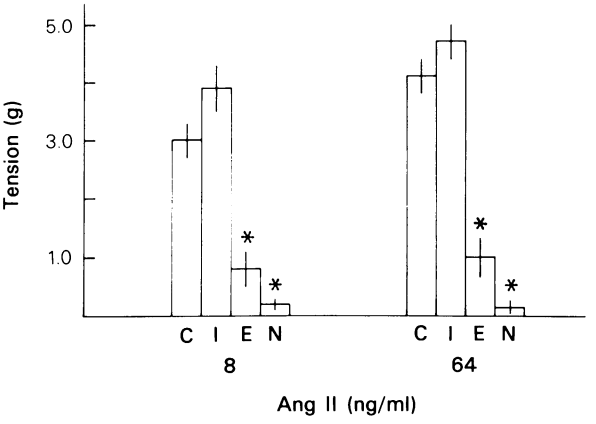

Figure 1 The effect of inhibitors of arachidonic acid conversion on angiotensin II (Ang II) induced contractions. C = control, I = indomethacin (2×10^{-6} M), E = ETYA (4×10^{-5} M) and N = nordihydroguaiaretic acid (10^{-5} M). The inhibitors were incubated for 5 min before testing the effect of Ang II. Statistical evaluation was $P < 0.001$ (*) and $P < 0.05$ (**); $n = 10$. The responses to Ang II in the presence of the synthesis inhibitors at 15 and 30 min were similar to the response at 5 min.

NDHGA also abolished the uterine contractile response to Ang II (Figure 1). At all time periods studied, NDHGA reduced the Ang II response almost to zero ($P < 0.001$). After washout of the lipoxygenase inhibitor, the response to Ang II did not return.

Pretreatment with indomethacin reduced the contractile response to low doses of oxytocin significantly (Figure 2). At higher doses of oxytocin the contrac-

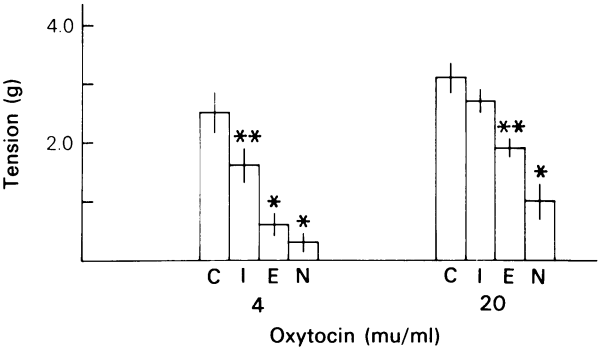

Figure 2 The effect of inhibitors of arachidonic acid conversion on oxytocin-induced contractions. C = control, I = indomethacin (2×10^{-6} M), E = ETYA (4×10^{-5} M), N = nordihydroguaiaretic acid (10^{-5} M). Statistical significance was $P < 0.001$ (*) and $P < 0.05$ (**); $n = 10$. The inhibitors were incubated for 5 min before testing the response to oxytocin. The results for 15 and 30 min were similar to the responses at 5 min.

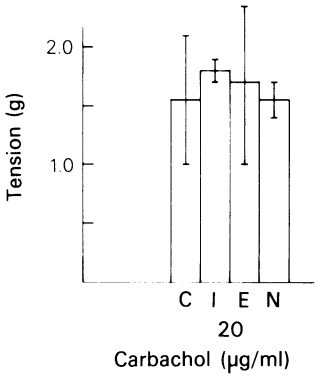

Figure 3 The effect of inhibitors of arachidonic acid conversion on carbachol-induced contractions. C = control, I = indomethacin (2×10^{-6} M), E = ETYA (4×10^{-5} M), N = nordihydroguaiaretic acid (10^{-5} M). Statistical evaluation of data showed non significant differences, $n = 6$. The prostaglandin synthesis inhibitors were incubated for 5 min before testing carbachol. The results at 15 and 30 min were similar to the results at 5 min.

tile response after indomethacin pretreatment was not significantly different from control values. At all incubation periods studied, 5, 15 or 30 min, the contractile response to oxytocin exhibited a similar pattern. After washing the tissue, the response to oxytocin was the same as the control.

Both ETYA and NDHGA reduced the contractile response to oxytocin (Figure 2). At 5, 15 or 30 min pretreatment, the effect of oxytocin was significantly reduced. Following ETYA pretreatment and replacing de Jalon's solution in the bath, the response to oxytocin returned to near control levels. However, after NDHGA pretreatment the response to oxytocin did not reach control values during the recovery period.

The contractile response to carbachol was unaltered by indomethacin, ETYA or NDHGA (Figure 3) at the various time periods studied.

Discussion

In the present study indomethacin did not abolish the Ang II response in the oestrogen-treated rat uterus, but did diminish the response to oxytocin at low doses of the peptide suggesting that prostaglandins are involved in this response. With respect to the response to Ang II after indomethacin, our findings are in contrast to those of Baudouin-Legros *et al.* (1974). These investigators found that indomethacin (5×10^{-6} M) suppressed the increased sensitivity of uterine tissue to Ang II and oxytocin in dioestrus and pro-oestrus but did not affect the response to prostaglandin $F_{2\alpha}$. Responses to Ang II during metoestrus were unmodified by indomethacin. However, these investigators did not test the action of indomethacin to the three agonists during oestrus.

Indomethacin has been shown to be an inhibitor of the cyclo-oxygenase pathway but not an inhibitor of the lipoxygenase cascade (Moncada & Vane, 1978). In fact, the product of the lipoxygenase pathway, 12L-hydroxy-5,8,10,14-eicosatetraynoic acid (HETE), was increased in the presence of indomethacin in platelets (Hamberg & Samuelsson, 1974). With indomethacin there appears to be a shift in the use of arachidonic acid to favour the products of the lipoxygenase pathway (Adcock & Garland, 1980). The abolition of the effects of indomethacin by lipoxygenase inhibitors supports this hypothesis (Adcock & Garland, 1980; Burka & Patterson, 1980).

Indomethacin has been reported to inhibit Ang II activity by acting as a possible receptor antagonist. Hall, Gurchinoff, Khosla & Khairallah (1978) found that indomethacin (10^{-5} M) and ETYA (10^{-5} M) reversed the antagonism of the Ang II analogue L-sarcosine, 8-threonine Ang II in rabbit isolated aorta while having no effect on the dose-response curves to Ang II. Investigating the possibility that indomethacin could affect the binding of Ang II, it was found that doses of indomethacin higher than 10^{-5} M reduced the binding capacity of tritiated Ang II to isolated adrenal cortical cells. Goodfriend & Simpson (1981), also found that indomethacin (10^{-5} to 10^{-4} M), meclofenamate (10^{-4} M) and ETYA (10^{-5} M) inhibited Ang II-induced contractions of bovine isolated umbilical artery as well as binding of Ang II to a particulate fraction at comparable doses.

The inhibitory effect of indomethacin on Ang II-induced contractions in guinea-pig isolated ileum did not appear to be related to the inhibition of prostaglandin synthesis (Aboulafia, Mendes, Miyamoto, Paiva & Paiva, 1976). These investigators found that indomethacin (56 μM) had an equal inhibitory effect on the contractile response to Ang II, bradykinin, histamine and acetylcholine, which may be due to inhibition of a common step in the excitation-contraction chain of events, such as translocation of calcium. These findings are supported by Northover (1971), who observed that widely different stimuli, such as electrical stimulation and histamine, were inhibited by very large concentrations of indomethacin. This author also presented evidence linking indomethacin inhibition to diminished calcium uptake by target cells.

From the present data there is no evidence that indomethacin acts in uterine tissue as a nonspecific receptor antagonist for Ang II. The possibility that indomethacin interferes with a common step in the excitation-contraction chain of events, is not substantiated by these data, since the contractile responses to

Ang II or carbachol were not affected by indomethacin. Further, stimulation of prostaglandin synthesis in the oestrogen-treated rat uterus is not a pre-requisite for stimulation of contractile activity by Ang II.

As a result of pretreatment with ETYA the contractile response in uterine smooth muscle to oxytocin and Ang II but not carbachol was diminished at all times studied. The inhibition of Ang II by ETYA may be due to inhibition of Ang II binding in a noncompetitive manner as suggested by Goodfriend & Simpson (1981). However, it is not possible from our data to distinguish between inhibition of binding and inhibition of prostaglandin synthesis.

Everitt, Spiegel, Bentley & Porter (1980) found that in the presence of indomethacin, arachidonic acid produced a slow, concentration-dependent contraction of guinea-pig isolated trachea which was antagonized by the lipoxygenase inhibitors NDHGA, ETYA and 3-amino-1-[*m*-(trifluoromethyl)phenyl]-2-pyrazoline. These authors suggest that the contractions to arachidonic acid appear to be mediated by the endogenous production of substances formed by the lipoxygenase pathway. Similarly, in the present study, contractions of uterine smooth muscle induced by Ang II and oxytocin but not by carbachol, were significantly diminished by NDHGA at all time periods studied. Thus if NDHGA is selectively inhibiting the lipoxygenase pathway in rat uterine smooth muscle, the peptide-induced contractions may be dependent upon the products of this pathway.

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