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Selective blockade of the vasodepressor response to prostaglandin $F_{2\alpha}$ in the anaesthetized rabbit

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Summary

1. The prostaglandin-blocking activity of meclofenamic acid, N-(2,6-dichloro-*m*-tolyl)anthranilic acid (CI-583), was analysed in the anaesthetized rabbit. $PGF_{2\alpha}$, PGE_1 and isoprenaline were injected before and after meclofenamic acid infusion.

2. Isoprenaline produced a fall in blood pressure, a reduction in oviduct motility and a reduction in uterine motility if the uterus showed marked spontaneous motility. $PGF_{2\alpha}$ uniformly produced a fall in blood pressure and an increase in both uterine and oviduct contractility. PGE_1 produced a fall in blood pressure, a reduction in oviduct motility and no consistent effect on uterine motility.

3. Meclofenamic acid selectively blocked the vasodepressor response to $PGF_{2\alpha}$. The vasodepressor responses to PGE_1 and isoprenaline as well as the effects of all three agonists on uterine and oviduct contractility were not reduced by treatment with meclofenamic acid.

4. Polyphloretin phosphate (PPP) administered to two rabbits in a cumulative dose of 94 mg/kg showed no significant blocking action on the vasodepressor or uterine and oviduct contractor responses to PGE_1 or $PGF_{2\alpha}$ though this compound, like meclofenamic acid, has been reported to antagonize the actions of PGE_1 and $PGF_{2\alpha}$ on isolated smooth muscle preparations.

Introduction

The biological activity of the prostaglandins is characterized by their ability to contract or relax smooth muscle. Many prostaglandins show both quantitative and qualitative differences in their effects on various smooth muscle systems. Horton & Main (1965) compared the actions of PGE_1 and $PGF_{2\alpha}$ on eight smooth muscle preparations. Both prostaglandins contracted intestinal smooth muscle in a variety of species as well as uterine smooth muscle in the rat. The rabbit oviduct, *in vivo*, was contracted by $PGF_{2\alpha}$ and relaxed by PGE_1. Both prostaglandins lowered arterial blood pressure in the anaesthetized cat and rabbit. These results suggest that there are several different types of prostaglandin receptors.

Further evidence for the existence of a prostaglandin receptor mechanism could be advanced if specific prostaglandin antagonists were available. Fried, Santhanakrishnan, Himizu, Lin, Ford, Rubin & Grigas (1969) reported that 9, 11--deoxy-7-oxaprostaglandin derivatives were able to reduce the contractor effects of PGE_1 in isolated tissue segments of the guinea-pig ileum, rabbit duodenum and

gerbil colon. Larger doses of these agents also reduced the contractor response to $PGF_{1\alpha}$ in the gerbil colon. Sanner (1969) found that 1-acetyl-2(8-chloro-10, 11-dihydrodibenz [b,f] [1,4] oxazepine-10-carbonyl) hydrazine (SC-19220) was a specific inhibitor of contractions induced by PGE_2 in the isolated guinea-pig ileum. In a series of publications (Eakins & Karim, 1970 ; Eakins, Karim & Miller, 1970 ; Eakins, Miller & Karim, 1971 ; Mathe, Strandberg & Astrom, 1971), polyphloretin phosphate (PPP) has been reported to be capable of blocking smooth muscle contractile responses to PGE_2 and $PGF_{2\alpha}$. Eakins, *et al.* (1971) obtained equivalent PA_2 values for polyphloretin phosphate in the isolated jird colon when PGE_2 and $PGF_{2\alpha}$ were used as agonists. This suggests that the same receptor is responsible for the contractor responses to both prostaglandins. Collier & Sweatman (1968) have reported the ability of fenamates, including meclofenamic acid to antagonize the contractor effects of $PGF_{2\alpha}$ and SRS-A on human isolated bronchial smooth muscle segments. However, in the *in vivo* Konzett-Rossler preparation of the guinea-pig lungs, meclofenamate did not block bronchoconstriction induced by $PGF_{2\alpha}$.

We have found that meclofenamic acid will specifically block the vasodepressor response to $PGF_{2\alpha}$ in the anaesthetized rabbit, using doses that do not reduce the effects of $PGF_{2\alpha}$ on uterine or oviduct motility. The vasodepressor, uterine and oviduct responses to isoprenaline and PGE_1 were not reduced.

Methods

Blood pressure, oviduct and uterine motility studies

Female rabbits, weighing 3·3–4·2 kg, were anaesthetized with pentabarbitone sodium (25 mg/kg) and barbitone sodium (100 mg/kg), injected together intraperitoneally. The trachea was cannulated. An external jugular vein was cannulated for intravenous injections. Blood pressure was recorded from a carotid artery with a Bourdon type pressure transducer (E & M Instrument Co.). The abdomen was opened with a midline incision and both uterine horns were located. A polyethylene catheter (inside diameter 0·5 mm, outside diameter 1 mm) was inserted through an incision in the ovarian end of one uterine horn so that its tip extended 0·5–1 cm into the isthmus of the oviduct. Another incision was made in the cervical end of the contralateral uterine horn and a catheter (inside diameter 1 mm, outside diameter 2 mm) was inserted so that its tip extended approximately 2 cm into the uterine cavity. Both catheters were carefully tied into place with minimal occlusion of the blood supply. Saline was perfused into the oviduct at a rate of 28 μl/min and into the uterus at the rate of 75 μl/min using separate perfusion pumps. A small incision was made in the tubal end of the perfused uterine horn to allow the perfusate to escape. A sidearm connected each perfusion syringe to a linear core pressure transducer (model P–1000, E & M Instrument Co.). All parameters were recorded with a Physiograph recorder (E & M Instrument Co.).

Drugs

$PGF_{2\alpha}$ and PGE_1 were dissovled in absolute alcohol in concentrations of 2 mg/ml from which appropriate dilutions with saline were prepared daily. Racemic isoprenaline was prepared as a 1 : 1,000 stock solution in saline containing 0·01%

ascorbic acid as a preservative. Appropriate dilutions were also prepared daily before use. Dilutions of all active drugs were kept on ice during each experiment. Meclofenamic acid (N–(2, 6-dichloro-*m*-tolyl)anthranilic acid, CI–583) and polyphloretin phosphate (PPP) were prepared as 1% solutions in 0·1N sodium hydroxide. Doses of isoprenaline are expressed in terms of the base.

Results

Effects of meclofenamic acid on oviduct, uterine and vasodepressor responses to PGF_2, PGE_1 and isoprenaline

Changes in uterine and oviduct perfusion pressure accurately reflect changes in tone in the circular muscle of the uterine horn and isthmus portion of the oviduct (Brundin, 1965). An increase in perfusion pressure is indicative of contraction; a decrease is indicative of relaxation. Six rabbits were used in this study. Isoprenaline was injected intravenously in a dose of 1 μg/kg, $PGE_{2\alpha}$ in a dose of 10 μg/kg and PGE_1 in a dose of 1 μg/kg. The ratio of $PGE_{2\alpha}$ to PGE_1

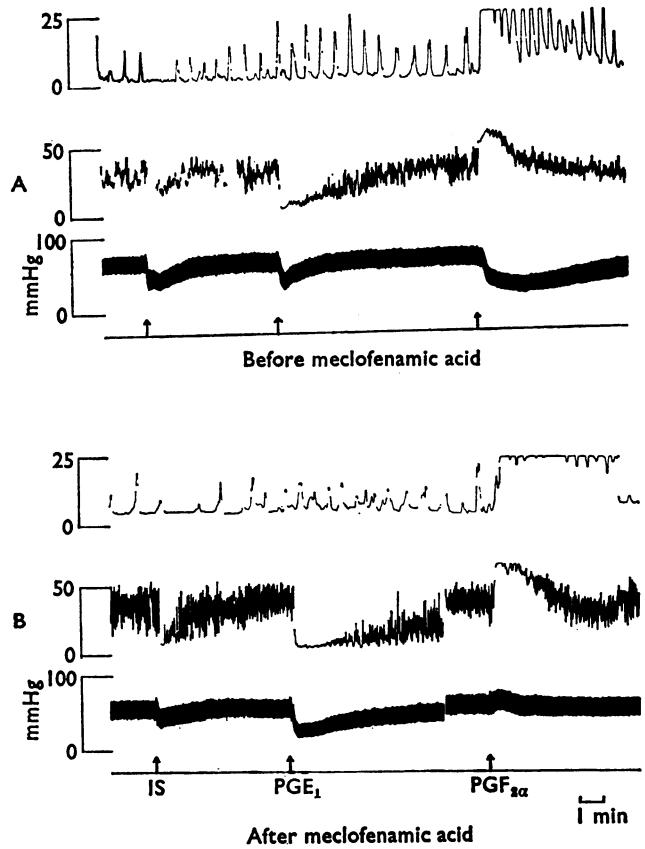

FIG. 1. Effects of meclofenamic acid on responses to isoprenaline, PGE_1 and $PGF_{2\alpha}$ in an anaesthetized rabbit. From above downward in each panel: uterine motility (mmHg), oviduct motility (mmHg) and arterial blood pressure (mmHg; 1 mmHg≡1·333 mbar). Isoprenaline (IS, 1 μg/kg), PGE_1 (1 μg/kg) and $PGF_{2\alpha}$ (10 μg/kg) before (panel A) and after (panel B) meclofenamic acid (30 mg/kg). All drugs injected intravenously.

dose of 10:1 was based upon the observation of Horton & Main (1965) of such a relative activity on rabbit blood pressure. All responses to the three agonists were submaximal. Isoprenaline uniformly evoked a fall in blood pressure, a reduction in oviduct motility and also a reduction of uterine motility if the uterus showed marked spontaneous motility. $PGF_{2\alpha}$ uniformly produced a fall in blood pressure, occasionally preceded by a small pressor response, and caused an increase in both uterine and oviduct motility in all rabbits. PGE_1 produced a fall in blood pressure and a reduction in oviduct motility but had no consistent effect on uterine motility. All agonists were administered to each rabbit before and after the intravenous infusion, over 20 min, of 30 mg/kg of meclofenamic acid. This dose of meclofenamic acid was determined as an effective dose after a number of preliminary experiments and was used in all six rabbits. The order of administration of the three agonists was randomized in order to eliminate the possibility of agonist interactions.

Figure 1 is typical of the results obtained in all six rabbits. None of the effects of isoprenaline and PGE_1 were reduced by meclofenamic acid. The effects of $PGF_{2\alpha}$ on uterine and oviduct motility were similarly unaffected by meclofenamic acid. On the other hand, the poent vasodepressor response to $PGF_{2\alpha}$ was markedly reduced and in three rabbits was reversed to a small pressor response. This $PGF_{2\alpha}$ 'reversal' phenomenon is clearly shown in Fig. 1B. The effects of meclofenamic acid on the vasodepressor responses to all agonists are summarized in Fig. 2. The increased vasodepressor response to PGE_1 as well as the slight decrease in the vasodepressor response to isoprenaline after meclofenamic acid, were not significantly different from their initial control values.

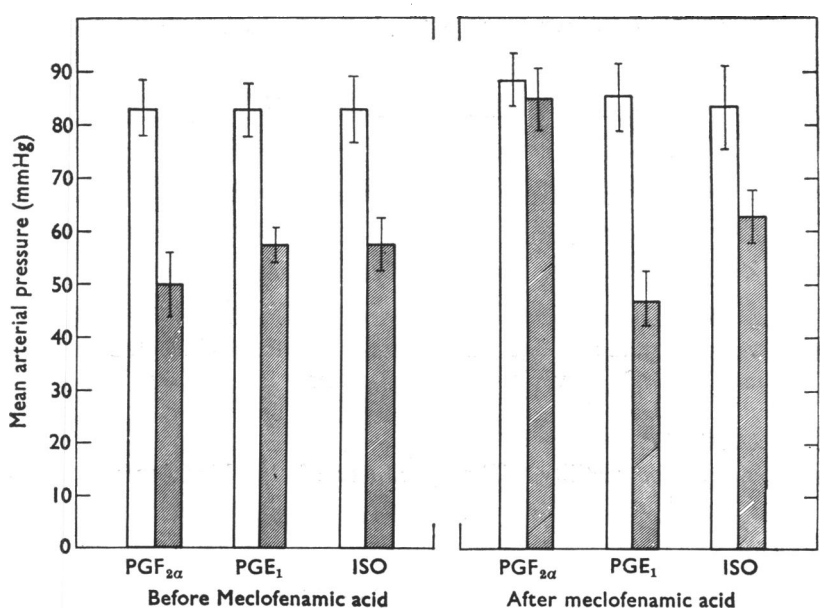

FIG. 2. Summary of the effects of meclofenamic acid pretreatment (30 mg/kg) on the vasodepressor responses to isoprenaline (ISO, 1 μg/kg), PGE_1 (1 μg/kg) and $PGF_{2\alpha}$ (10 μg/kg). Blank bars represent preinjection control mean arterial blood pressure levels. Cross-hatched bars represent mean arterial blood pressure values at the peak of the vasodepressor response. All results represent the mean values $\pm$ S.E. from six rabbits.

The vasodepressor responses to $PGF_{2\alpha}$ were selectively blocked by meclofenamic acid.

In three of the six rabbits, three successive injections of $PGF_{2\alpha}$ were administered at 15–20 min intervals. This was done in order to rule out the possibility that the reduction in the vasodepressor response to $PGF_{2\alpha}$ after meclofenamic acid might simply have been due to the production of tachyphylaxis. No diminution in responsiveness to the drug was observed. Also, two additional rabbits were given $PGF_{2\alpha}$ (10 μ/kg) at 30 min intervals over 3 h with no significant reduction in the vasodepressor response from its initial control value.

Effects of polyphloretin phosphate (PPP) on $PGF_{2\alpha}$ and PGE_1 responses

PPP, like meclofenamic acid, blocks contractile responses induced by $PGF_{2\alpha}$ in isolated segments of human bronchi (Mathe *et al.*, 1971). PGE_1 and $PGF_{2\alpha}$, in doses of 1 and 10 μg/kg respectively, were administered before and after successive doses of 1, 3, 10, 30 and 50 mg/kg of PPP (cumulative dose=94 mg/kg) to two rabbits. PPP treatment did not significantly inhibit the actions of either PGE_1 or $PGF_{2\alpha}$ on blood pressure, uterine or oviduct motility. On the basis of these results obtained in two rabbits, PPP appeared to be devoid of prostaglandin blocking activity.

Discussion

The possibility that the prostaglandins may exert their effects by activation of several different types of receptors is receiving growing support. Horton & Main (1965) noted that PGE_1 was more potent than $PGF_{2\alpha}$ in eliciting similar inhibitory effects while $PGF_{2\alpha}$ was more potent than PGE_1 in eliciting similar excitatory effects. Since differing orders of potency was the basis used by Ahlquist (1948) for the classification of adrenergic receptors into α and β subtypes, this is a significant observation. Adamson, Eliasson & Wiklund (1967) noted that after the isolated rat uterus developed a decreasing sensitivity to PGE_1 or $PGF_{2\alpha}$ it still responded normally to the other prostaglandin. They suggest that these two similar effects might be due to different receptors. The possibility of two closely related drugs chemically eliciting similar responses through different receptors in the same tissue is unusual but not impossible. Intestinal smooth muscle has both α and β-adrenoceptors and both receptors subserve inhibition (Levy & Ahlquist, 1967).

The classification of prostaglandin receptor mechanisms would be further supported by the ability of a specific antagonist or antagonists to produce selective blockade of any of the effects of the various prostaglandins. This study demonstrates the ability of meclofenamic acid to produce a selective blockade of the vasodepressor response to $PGF_{2\alpha}$ in a dose that did not reduce the effects of $PGF_{2\alpha}$ on either uterine or oviduct contractility. The specificity of this blocking effect is further supported by the inability of meclofenamic acid to reduce the vasodepressor response to isoprenaline. Since isoprenaline exerts its vasodepressor effect by activating inhibitory β-adrenoceptors in vascular smooth muscle, this was not an unexpected finding. The fact that meclofenamic acid also did not reduce the vasodepressor response to PGE_1 is interesting. This would suggest the possibility of PGE_1 and $PGF_{2\alpha}$ eliciting vasodepressor responses through different receptor mechanisms.

The results of this limited study establishing a selective blockade of a single parameter of $PGF_{2\alpha}$ *in vivo* do not warrant the classification of a prostaglandin receptor mechanism at this time. The observations, however, are indicative of the complex relationships that may be expected should the prostaglandins indeed act through a multiple receptor mechanism.

The preliminary studies conducted with polyphloretin phosphate (PPP) suggest that this compound is devoid of the type of prostaglandin blocking activity demonstrated by meclofenamic acid in the rabbit, despite the ability of both compounds to block contractor responses induced by $PGF_{2\alpha}$ in isolated human bronchial segments.

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