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A COMPARISON OF THE ACTIONS OF PROSTAGLANDINS $F_{2\alpha}$ AND E_1 ON SMOOTH MUSCLE

BY

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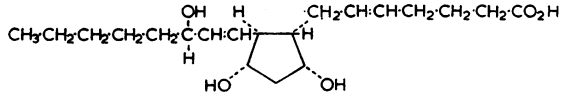
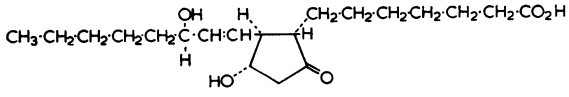
In a previous investigation we compared the biological activities of four prostaglandins, E_1, E_2, E_3 and $F_{1\alpha}$ (Horton & Main, 1963). Prostaglandins of the E series were qualitatively very similar in their effects and their ratios of activity were approximately constant on all the biological preparations tested. In contrast, the biological activity of prostaglandin $F_{1\alpha}$ relative to E_1 varied widely on different preparations, and on one, the rabbit fallopian tube intraluminal pressure, the two prostaglandins produced opposite effects.



Prostaglandin $F_{2\alpha}$: 9α,11α,15-trihydroxy-prost-5,13-dienoic acid



Prostaglandin E_1: 11α,15-dihydroxy-9-oxo-prost-13-enoic acid

Fig. 1. Formulae of prostaglandin $F_{2\alpha}$ and prostaglandin E_1

In this investigation we have compared a fifth prostaglandin, $F_{2\alpha}$ (Fig. 1), directly with prostaglandin E_1 on eight smooth muscle preparations selected for their known sensitivity to E_1. We have found that, on preparations which are inhibited by prostaglandins, $F_{2\alpha}$ is always less active than E_1, but that, on preparations which are contracted, $F_{2\alpha}$ is usually more active than E_1. These findings are of interest since $F_{2\alpha}$ is the prostaglandin which predominates in several tissues (Bergström, 1964).

METHODS

Smooth muscle preparations in vitro

Segments of various organs were suspended in a 4- or 10-ml. organ-bath. Longitudinal contractions were recorded either isotonically with a frontal-writing lever on a smoked drum, or isometrically with a

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force-displacement transducer and an ink-writing polygraph. A dose cycle of 4 to 6 min with a contact time of 45 to 90 sec was used for all preparations except the cat trachea.

Rabbit jejunum. Proximal jejunum, from rabbits weighing 2 to 3 kg, was suspended in Tyrode solution at 35 to 36° C, gassed with air.

Guinea-pig ileum. Terminal ileum, from guinea-pigs weighing 200 to 450 g, was suspended in Tyrode solution at 37° C, gassed with air.

Hamster colon. The ascending colon, from hamsters weighing 100 to 200 g, was suspended in de Jalon solution at 35 to 36° C, gassed with air.

Cat trachea. Tracheas were obtained from cats which had been anaesthetized with sodium pentobarbitone, and which had been used for other experiments. Two or three rings of trachea were tied together with the muscle in alignment; the cartilage was removed. The preparations were suspended in Krebs-Henseleit solution at 37° C, gassed with 95% oxygen and 5% carbon dioxide.

Rat uterus. Segments of uterus from rats weighing 150 to 250 g, which had been injected subcutaneously with stilboestrol (100 μg) 18 hr previously, were suspended in de Jalon at 32° C, gassed with air.

Smooth muscle preparations in vivo

Rabbit blood pressure and fallopian tube intraluminal pressure. Rabbits weighing 2 to 3 kg were anaesthetized with urethane (1.75 g/kg) injected intraperitoneally. Arterial blood pressure and fallopian tube intraluminal pressure were recorded with pressure transducers as described previously (Horton & Main, 1963).

Cat gastrocnemius muscle blood flow. In one cat weighing 3.4 kg gastrocnemius muscle blood flow was recorded as described previously (Horton & Main, 1963). Prostaglandins were injected intra-arterially.

RESULTS

In all experiments the relative activity of the two prostaglandins was estimated by bracketing. Threshold doses quoted for prostaglandin $F_{2\alpha}$ are approximate. The results are summarized in Table 1.

TABLE 1

APPROXIMATE THRESHOLD DOSES OF PROSTAGLANDIN $F_{2\alpha}$ AND ITS BIOLOGICAL ACTIVITY RELATIVE TO PROSTAGLANDIN E_1

In three out of four experiments prostaglandin $F_{2\alpha}$ increased the tone of the fallopian tubes, in contrast to prostaglandin E_1 which caused relaxation. Relative activities are means, with ranges in parentheses

Preparation	Response	Threshold dose (ng/ml. *in vitro*) (μg/kg *in vivo*)	Relative activity, $F_{2\alpha}$ to E_1	No. of experiments: Qualitative difference between $F_{2\alpha}$ and E_1	No. of experiments: Total
Rabbit jejunum	Contraction	0·25–1	26 (5–100)	5	7
Guinea-pig ileum	Contraction	10–50	0·55 (0·015–3)	3	7
Hamster colon	Contraction	1–5	3·5 (1–8)	0	5
Cat trachea	Relaxation	50–1,000	0·03 (0·0025–0·05)	0	4
Rat uterus	Contraction	25–50	8 (5–15)	0	4
Rabbit fallopian tube	Contraction	1·3–5	—	3	4
Rabbit blood pressure	Depression	0·7–2·5	0·11 (0·08–0·2)	3	4
Cat gastrocnemius muscle blood flow	Vasodilatation		<0·05	0	1

Intestinal smooth muscle

The rabbit isolated jejunum contracted in response to prostaglandin $F_{2\alpha}$ in concentrations of 0.25 ng/ml. In five out of seven experiments there was a qualitative difference in the responses to prostaglandin $F_{2\alpha}$ and prostaglandin E_1. The contraction due to prostaglandin $F_{2\alpha}$ was slower in onset and reached a maximum more slowly than the contraction to prostaglandin E_1 (Fig. 2). This qualitative difference made meaningful quantitative com-

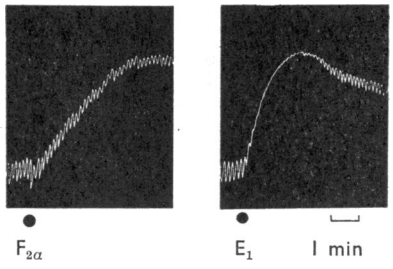

Fig. 2. Isotonic responses of isolated rabbit jejunum preparation suspended in 4-ml. organ-bath containing Tyrode solution. $F_{2\alpha}$=prostaglandin $F_{2\alpha}$, 4 ng/ml.; E_1=prostaglandin E_1, 20 ng/ml.

parisons difficult. Prostaglandin $F_{2\alpha}$ was always more active than prostaglandin E_1, the amount varying from 5- to 100-times with different intestines. The biggest differences were found in preparations which were less sensitive than usual to prostaglandin E_1 although the sensitivity to prostaglandin $F_{2\alpha}$ was normal. The reason for this discrepancy is unexplained. It could not be accounted for by deterioration in the prostaglandin solutions because freshly prepared solutions were also less active on these less sensitive preparations.

A similar qualitative difference between the prostaglandins was sometimes observed (three out of seven experiments) on the guinea-pig isolated ileum. On this preparation the ratio of activity of E_1 to $F_{2\alpha}$ was even more variable than on the rabbit jejunum. On average, prostaglandin E_1 was slightly more active but the relative activities varied from 0.015 to 3.0 on different preparations.

No qualitative differences in the stimulant action of the two prostaglandins were observed on the hamster isolated colon. In four out of five experiments prostaglandin $F_{2\alpha}$ was more active than E_1, in the remaining experiment they were equiactive.

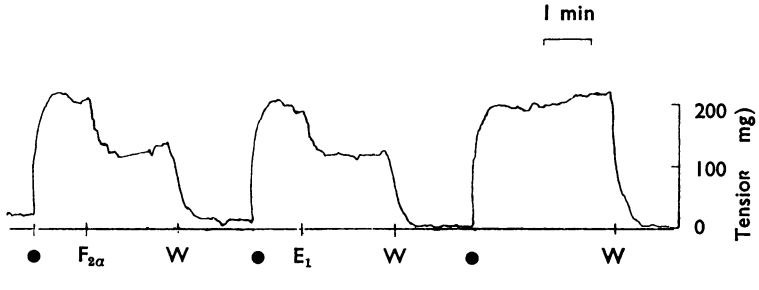

Fig. 3. Isometric responses of cat isolated trachea, suspended in 4-ml. organ-bath containing Krebs-Henseleit solution. At the dots acetylcholine (12.5 ng/ml.) was added. $F_{2\alpha}$=prostaglandin $F_{2\alpha}$, 0.75 μg/ml.; E_1=prostaglandin E_1, 1.9 ng/ml.; W=wash.

Respiratory smooth muscle

The cat isolated tracheal chain has little or no inherent tone; inhibitory responses are therefore difficult to detect. If a contraction of the preparation is produced by acetylcholine, prostaglandins can be shown to have inhibitory effects (Main, 1964). Using this technique, we have shown that, like prostaglandin E_1, $F_{2\alpha}$ also inhibits this preparation. The activity of prostaglandin $F_{2\alpha}$ was, however, only about one-thirtieth of that of E_1 (Fig. 3). In view of the small amounts of prostaglandin $F_{2\alpha}$ available, comparisons were not made on other preparations of respiratory smooth muscle which are affected by prostaglandin E_1 (Main, 1964).

Reproductive smooth muscle

Both prostaglandins contracted the isolated uterus of the rat, prostaglandin $F_{2\alpha}$ being more active than E_1. The threshold concentration of prostaglandin $F_{2\alpha}$ was about 25 ng/ml. The rabbit fallopian tube *in vivo*, which is relaxed by small doses of prostaglandin E_1, was

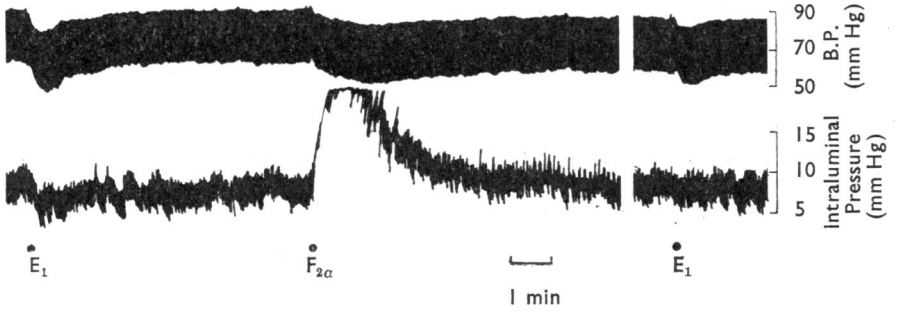

Fig. 4. Record of rabbit (2.8 kg) blood pressure (B.P., upper trace) and fallopian tube intraluminal pressure (lower trace). $F_{2\alpha}$=prostaglandin $F_{2\alpha}$, 10 μg; E_1=prostaglandin E_1, 2 and 1 μg at first and second injections respectively.

contracted by prostaglandin $F_{2\alpha}$ in three out of four experiments (Fig. 4). In the fourth experiment prostaglandin $F_{2\alpha}$ produced a short-lasting relaxation. Prostaglandin $F_{2\alpha}$ was much less active than E_1 on this preparation, the threshold dose being about 5 μg/kg.

Vascular smooth muscle

On intravenous injection into the anaesthetized rabbit both prostaglandins caused a fall in arterial blood pressure. Prostaglandin $F_{2\alpha}$ was about one-tenth as active as prostaglandin E_1. In three out of four experiments prostaglandin $F_{2\alpha}$ produced a more gradual and more prolonged fall of pressure than occurred with prostaglandin E_1 (Fig. 4). In one experiment on the cat gastrocnemius muscle blood flow, prostaglandin $F_{2\alpha}$ was shown to have vasodilator activity, but it was less active than prostaglandin E_1. In view of this low activity and of the need to conserve supplies of prostaglandin $F_{2\alpha}$ no further blood flow experiments were carried out.

DISCUSSION

Prostaglandins E_1, E_2 and E_3 differ chemically only in their degree of unsaturation and have very similar biological activities; E_3 is usually the least potent (Horton & Main, 1963; Bergström & Euler, 1963; Horton, 1964; Main, 1964). On the other hand, prostaglandin $F_{1\alpha}$, which can be formed by the reduction of the oxo substituent in the cyclopentane ring of prostaglandin E_1, differs more significantly from prostaglandins of the E series (Horton & Main, 1963). It could be predicted that the introduction of a second double bond in the prostaglandin $F_{1\alpha}$ molecule would have a similar effect upon its biological activity as a change from prostaglandin E_1 to prostaglandin E_2, and that, in view of the similarity of E_1 and E_2, the two prostaglandin Fs would also have very similar activity. How far is this prediction substantiated by the experimental findings? In our previous investigation we estimated the relative activities of E_2 to E_1 and of $F_{1\alpha}$ to E_1; the expected relative activities of $F_{2\alpha}$ to E_1 can therefore be calculated. The general trend of the predictions is supported

TABLE 2

BIOLOGICAL ACTIVITY OF PROSTAGLANDIN $F_{2\alpha}$ RELATIVE TO PROSTAGLANDIN E_1

Results predicted from previous studies are compared with observed results. * Prostaglandin $F_{1\alpha}$ and $F_{2\alpha}$ increased the tone of the fallopian tubes in contrast to prostaglandins of the E series which caused relaxation

	Relative biological activity				
Preparation	Observed E_2/E_1	Observed $F_{1\alpha}/E_1$	Predicted $F_{2\alpha}/E_1$	Observed $F_{2\alpha}/E_1$	Calculated $F_{2\alpha}/F_{1\alpha}$
Rabbit jejunum	1·5	2·2	3·3	26	12
Guinea-pig ileum	1·6	0·02	0·03	0·55	27
Hamster colon	2·8	0·26	0·73	3·5	13
Cat trachea	1·0	0·002	0·002	0·03	15
Rat uterus	1·1	0·9	1·0	8	9
Rabbit fallopian tube	0·9	*	*	*	*
Rabbit blood pressure	1·0	0·08	0·08	0·1	1·25
Cat gastrocnemius muscle blood flow	0·8	0·2	0·16	<0·05	0·3

by the experimental results (Table 2). Thus $F_{2\alpha}$ has weak inhibitory actions on cat isolated trachea and on vascular smooth muscle, while on the rabbit fallopian tube prostaglandin $F_{2\alpha}$, like $F_{1\alpha}$ but unlike E_1, increases intraluminal pressure. However, on all tissues (except vascular smooth muscle) the activity of $F_{2\alpha}$ was some ten times greater than predicted. The reason for this apparent discrepancy cannot be explained. Anggård & Bergström (1963) also found that $F_{2\alpha}$ is more potent than $F_{1\alpha}$ but they made no direct comparison. We have been able to confirm other observations of Anggård & Bergström (1963), namely that the rabbit isolated duodenum or jejunum is very sensitive to $F_{2\alpha}$ and that $F_{2\alpha}$ is not a very potent depressor substance but that like other prostaglandins it does increase blood flow through skeletal muscle. We were unable to confirm that the rat isolated uterus will respond to $F_{2\alpha}$ in concentrations as low as 1 ng/ml. although it is more active than other prostaglandins on this preparation. It is possible that the exact stage in the oestrous cycle is critical for such high sensitivity.

One important reason for comparing the activities of different prostaglandins is to establish which tissue is most suitable for the assay of a particular prostaglandin and

which pair of tissues might be used to distinguish between two prostaglandins or to estimate their concentrations in a mixture. Biological methods of estimation are more sensitive than any known chemical methods and are the only means of assaying the minute quantities found in small amounts of tissue. We have previously concluded that no combination of biological preparations yet known will distinguish between the three prostaglandin Es but that $F_{1\alpha}$ and E_1 could be distinguished by parallel assays on rabbit jejunum and guinea-pig ileum (Horton & Main, 1963) or rabbit jejunum and cat trachea (Main, 1964). The rabbit jejunum and cat trachea could also be used to distinguish between the prostaglandin Es and prostaglandin $F_{2\alpha}$ (index of discrimination=800). It is doubtful whether any pair of the tissues tested in this investigation would enable us to distinguish between prostaglandin $F_{1\alpha}$ and prostaglandin $F_{2\alpha}$. A combination of, say, guinea-pig ileum and rabbit blood pressure might provide a high enough index of discrimination. However, the rabbit blood pressure is too insensitive for use as a routine assay method where only nanograms of prostaglandins may be present in the sample to be assayed.

In addition to a qualitative difference in the response of the rabbit fallopian tube, $F_{2\alpha}$ and E_1 differed in the type of response on the rabbit blood pressure and certain types of isolated intestinal smooth muscle. On both rabbit blood pressure and rabbit isolated jejunum the responses to $F_{2\alpha}$ were usually slower in onset and took longer to reach a maximum than the responses to E_1. Similar differences were not observed between prostaglandin $F_{1\alpha}$ and prostaglandin E_1 in our previous investigation, but Bergström, Eliasson, Euler & Sjövall (1959) did report that the response to $F_{1\alpha}$ was slower than that to E_1. It is unlikely that the slower response is due to conversion of prostaglandin $F_{2\alpha}$ to E_2 since $F_{2\alpha}$ is active at lower concentrations than E_2. Possibly it is related to the ease with which this compound is transported to the active site.

One of the most interesting features which has emerged from this investigation is the contrast in potency of prostaglandin $F_{2\alpha}$ as an inhibitor and as a stimulator of smooth muscle. It had already become apparent from our previous work (Horton & Main, 1963; Main, 1964) that $F_{1\alpha}$ is less potent than E_1 on smooth muscle which is inhibited and the present results suggest that this is a general property of prostaglandin Fs. In some respects the difference is similar to that existing between adrenaline and noradrenaline. Prostaglandin E_1, like adrenaline, is potent on tissues which are inhibited, whereas both catechol amines and both prostaglandins are potent as stimulators. It is perhaps premature to speculate about two kinds of prostaglandin receptor.

Information about the distribution of the different prostaglandins in different tissues is still incomplete. Prostaglandin $F_{2\alpha}$ appears to be more widely distributed than the others; it has been isolated from lung, brain, iris, semen and menstrual fluid. In view of its presence in lung and brain it is of interest that it is considerably less active than the corresponding prostaglandin E in relaxing respiratory smooth muscle and in causing stupor and sedation (Horton & Main, 1965). If prostaglandins are concerned in the control of smooth muscle tone in the lungs (Main, 1964), it would seem likely that prostaglandin E_2 rather than prostaglandin $F_{2\alpha}$ would be of more immediate physiological importance. Possibly prostaglandin $F_{2\alpha}$ in lung and brain represents a tissue store of prostaglandin which is either a relatively inactive metabolite of prostaglandin E_2 or a precursor which exerts physiological effects only after conversion to prostaglandin E_2.

SUMMARY

1. The biological activities of four prostaglandins have previously been reported; in the present paper the activity of a fifth prostaglandin, $F_{2\alpha}$, is compared with prostaglandin E_1 on smooth muscle preparations.

2. Both prostaglandins contract intestinal smooth muscle of the rabbit, hamster and guinea-pig *in vitro*. Responses of the rabbit jejunum to prostaglandin $F_{2\alpha}$ are usually slower in onset and more prolonged than those to prostaglandin E_1.

3. Both prostaglandins inhibit acetylcholine-induced contractions of the cat isolated tracheal chain, but $F_{2\alpha}$ has only one-thirtieth of the activity of E_1.

4. The rat isolated uterus is contracted by both prostaglandins, but is more sensitive to $F_{2\alpha}$. On the other hand, the rabbit fallopian tube *in vivo*, which is relaxed by small doses of prostaglandin E_1, is contracted by $F_{2\alpha}$.

5. Both prostaglandins increase blood flow through skeletal muscle in the cat and lower arterial blood pressure; $F_{2\alpha}$ is less potent in this respect than E_1.

6. It is concluded that prostaglandin $F_{2\alpha}$ is a less potent inhibitor of smooth muscle than prostaglandin E_1, but that, on smooth muscle preparations which are contracted, $F_{2\alpha}$ is often more active than E_1.

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