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THE INHIBITORY ACTIONS OF PROSTAGLANDINS ON RESPIRATORY SMOOTH MUSCLE

BY

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Prostaglandin E_1, in concentrations as low as 1 ng/ml., relaxed isolated tracheal muscle from cat, monkey, rabbit, guinea-pig and ferret. Tracheal muscle from the cat, monkey and rabbit did not exhibit inherent tone and the effect of prostaglandin E_1 on these preparations was seen only after a sustained contraction had been produced by a previous dose of acetylcholine or of another agonist. Prostaglandins E_2, E_3 and $F_{1\alpha}$ also relaxed isolated cat tracheal muscle which had been stimulated by acetylcholine: their activities relative to that of prostaglandin E_1 were, respectively, 1.0, 0.2 and 0.002. In the anaesthetized cat prostaglandin E_1 increased lung "resistance to inflation" (presumably comparable to bronchial resistance) and the heart rate. In the anaesthetized rabbit and guinea-pig, prostaglandin E_1 antagonized the rise in resistance to inflation of the lungs obtained after vagal stimulation or after the intravenous injection of histamine; it sometimes lowered the resistance to inflation in these species. The possibility that prostaglandin may have a local physiological role in the control of bronchial smooth muscle tone is discussed.

Prostaglandin was first discovered in semen and in the prostate gland (Goldblatt, 1933; Euler, 1934). Recently six different prostaglandins have been isolated from various mammalian tissues (Bergström, Ryhage, Samuelsson & Sjövall, 1962; Bergström, Dressler, Ryhage, Samuelsson & Sjövall, 1962; Bergström, Dressler, Krabisch, Ryhage & Sjövall, 1962). The lungs, notably, of all the species examined contain prostaglandins, mainly prostaglandin $F_{2\alpha}$ (Bergström *et al.*, 1962a) with small amounts of prostaglandin $F_{3\alpha}$ and E_2. From experiments to test the effect of prostaglandin $F_{2\alpha}$ on respiratory smooth muscle Änggård & Bergström (1963) concluded that it increased bronchial resistance in the cat, but was inactive on isolated cat tracheal muscle.

In the investigation described here prostaglandins E_1, E_2, E_3 and $F_{1\alpha}$ have been found to relax tracheal muscle *in vitro* and, except in the cat, to decrease lung "resistance to inflation" *in vivo*.

METHODS

Tracheal muscle preparations in vitro

Rabbit, guinea-pig, ferret, pig and sheep tracheas were obtained from freshly stunned and bled animals. Cat tracheas were obtained after the cats had been decerebrated during anaesthesia with ether for other purposes. Monkey tracheas were obtained from animals killed by intravenous injection of pentobarbitone sodium.

Two to eight tracheal rings were tied together with the muscle strips in alignment, and the cartilage was removed. Preparations were suspended in Krebs-Henseleit solution in a 4 or 10 ml. organ-bath at 37° C, bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Contractions were recorded isotonically using a frontal writing lever and a smoked drum. The magnification ranged from twenty-times with a load of 0.3 g for guinea-pig tracheas, to five times with a load of 2.5 g for pig and sheep tracheas.

" Resistance to inflation " of the lungs in vivo

Cats weighing 2.3 to 3.2 kg were anaesthetized with chloralose (80 mg/kg) and pentobarbitone sodium (6 mg/kg), or with chloralose (50 mg/kg) and urethane (0.5 g/kg), injected intraperitoneally. Rabbits weighing 2.6 to 3.0 kg and guinea-pigs weighing 250 to 500 g were anaesthetized with urethane (1.75 g/kg).

Blood pressure and heart rate were recorded from a carotid artery using a Statham pressure transducer (P23AC) and a heart rate meter. Left or right vagus nerves were stimulated with rectangular pulses (2.5 V, 0.5 msec duration, 10 shocks/sec). Drugs were injected through a cannula in an external jugular vein.

Changes in " resistance to inflation " of the lungs were assessed by the method of Konzett & Rössler (1940). The trachea was cannulated and in some experiments the chest was opened widely. The lungs were ventilated by a Starling pump at an inflow pressure of 5 to 10 cm of water. " Overflow air," which did not enter the lungs, passed into a 1 l. bottle connected to a volumetric pressure transducer (Statham PT5A). In some experiments on guinea-pigs, a lever mounted on a rubber diaphragm and writing on a smoked drum was used.

Drugs

These were acetylcholine hydrochloride, histamine acid phosphate (B.D.H.) and dihydroergotamine methanesulphonate (Sandoz). All concentrations are expressed in terms of the bases.

RESULTS

Isolated tracheal muscle preparations

Acetylcholine contracted tracheal muscle of all the species tested. The concentrations (μg/ml.) usually required for a response in the range 50 to 75% of the maximum were: cat, 0.5 to 5; monkey, 1 to 10; rabbit, 0.5 to 5; ferret, 0.25 to 1; guinea-pig, 0.1 to 5; sheep, 0.25 to 2.5; and pig, 1 to 5 μg/ml.

Effect of prostaglandin E_1 on tracheal contractions due to acetylcholine. The inhibitory action of prostaglandin E_1 on cat tracheal muscle was demonstrated in two ways. When prostaglandin E_1 was added to the organ-bath shortly before acetylcholine, the response to acetylcholine was reduced compared with contractions obtained in the absence of prostaglandin. When prostaglandin was added to the bath after a dose of acetylcholine had produced a sustained contraction, the muscle relaxed. These effects are illustrated in Fig. 1, when 0.125 μg/ml. of prostaglandin E_1 caused 30 to 40% inhibition of the response of the cat isolated trachea preparation to acetylcholine (0.5 μg/ml.).

The threshold concentration of prostaglandin E_1 which relaxed the cat isolated trachea was as low as 1 ng/ml. Concentrations of 0.5 μg/ml. or less inhibited the effect of acetylcholine by 95% in several experiments. Tracheal muscle from the monkey was almost as sensitive as that of the cat (Fig. 2, *a*). Prostaglandin E_1 (0.5 to 4 μg/ml.) inhibited by 50% the response of the rabbit trachea to acetyl-

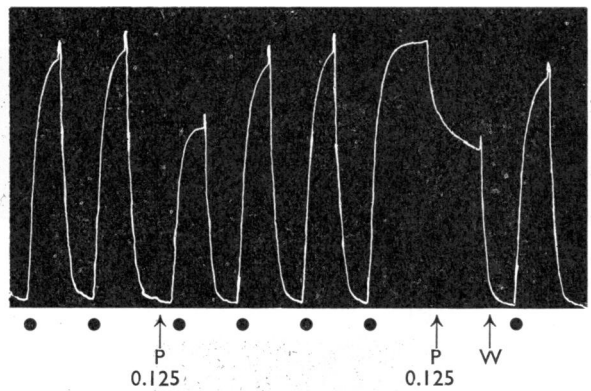

Fig. 1. Responses of a cat isolated trachea preparation, suspended in a 4 ml. organ-bath containing Krebs–Henseleit solution. At the dots acetylcholine (0.5 μg/ml.) was added. P=prostaglandin E_1 (0.125 μg/ml.); W=wash.

choline. Complete inhibition was not observed even with higher concentrations (Fig. 2, *b*).

Guinea-pig and ferret tracheas possessed inherent tone *in vitro*. Threshold concentrations of 0.005 to 0.1 μg/ml. of prostaglandin E_1 reduced tone and antagonized acetylcholine (Fig. 2, *c*). Sheep and pig tracheas usually possessed tone *in vitro*. On the pig trachea prostaglandin E_1 antagonized acetylcholine but had no effect on tone, while in two out of three experiments on sheep tracheas, prostaglandin E_1 in concentrations of 5 μg/ml. had no effect on the response to acetylcholine or on tone; in the third experiment slight inhibition was observed with 3 μg/ml. of prostaglandin E_1.

Effect of prostaglandins E_2, E_3 and $F_{1\alpha}$ on tracheal contractions due to acetylcholine. Prostaglandins E_2, E_3 and $F_{1\alpha}$ antagonized acetylcholine on the cat isolated trachea preparation. Their activities expressed relative to E_1 were 1.0, 0.2 and 0.002 respectively.

Effect of prostaglandin E_1 on tracheal contractions due to histamine. Although histamine, in concentrations of 10 to 100 μg/ml., did not contract tracheal muscle from the cat, monkey, rabbit or pig, these doses often antagonized acetylcholine. In one experiment on the ferret trachea histamine at low concentrations (0.5 μg/ml.) caused small contractions, but at higher concentrations (2 to 10 μg/ml.) it caused relaxation. In this respect histamine was at least 500-times less potent than prostaglandin E_1.

Responses of the guinea-pig trachea to histamine were antagonized by prostaglandin E_1 in concentrations as low as 5 ng/ml.

Effect of prostaglandin E_1 on tracheal contractions due to dihydroergotamine. In concentrations of 5 to 20 μg/ml., dihydroergotamine caused a slow contraction in six of the eleven cat tracheal preparations tested. The remaining preparations did not respond to concentrations as high as 250 μg/ml. Contractions reached a maximum in 15 to 20 min and were maintained for several hours in spite of repeated washing.

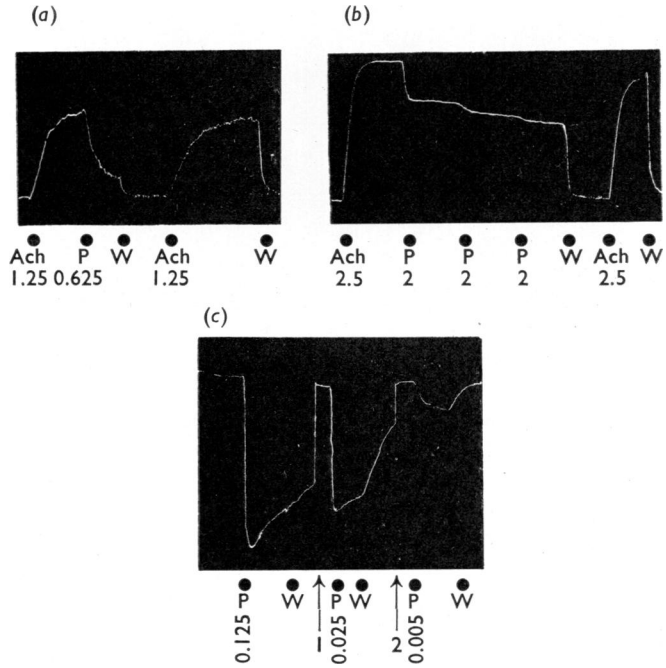

Fig. 2. Responses of isolated trachea preparations suspended in 4 ml. organ-baths containing Krebs–Henseleit solution. (*a*), monkey; (*b*), rabbit; and (*c*), ferret. Ach=acetylcholine; P=prostaglandin E_1; W=wash. All drug concentrations are in μg/ml. At arrows 1 and 2, the drum was stopped for 20 and 10 min respectively.

Dihydroergotamine had no effect on tracheal preparations from the rabbit, ferret, pig or sheep, or on preparations from the monkey in three out of four experiments In the fourth experiment on monkey trachea, 100 μg/ml. of dihydroergotamine caused a small contraction which was not maintained after washing. Slight relaxation was observed with 100 μg/ml. on the guinea-pig tracheal preparation.

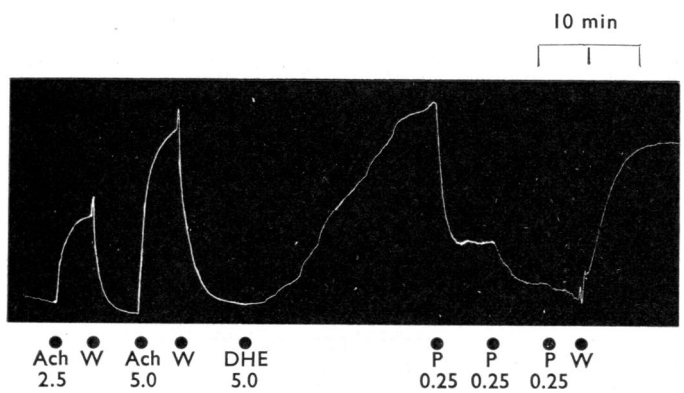

Fig. 3. Responses of cat isolated trachea preparation, suspended in a 4 ml. organ-bath containing Krebs–Henseleit solution. Ach=acetylcholine; DHE=dihydroergotamine; P=prostaglandin E_1; W=wash. All drug concentrations are in μg/ml.

Prostaglandin E_1, in concentrations as low as 1 ng/ml., antagonized dihydroergotamine. Fig. 3 shows a partial inhibitory effect of prostaglandin E_1 (0.25 μg/ml.) on a contraction produced by dihydroergotamine (5 μg/ml.). A further dose of prostaglandin E_1 caused almost complete relaxation of the muscle followed by recovery of tone on washing.

Effect of prostaglandin E_1 on tracheal contractions due to barium chloride. Barium chloride, in concentrations of 0.1 to 1.0 mg/ml., caused a slow contraction

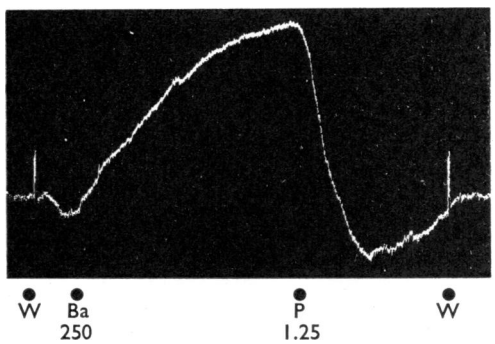

Fig. 4. Responses of a guinea-pig isolated trachea preparation, suspended in a 4 ml. organ-bath containing Krebs–Henseleit solution. Ba=barium chloride; P=prostaglandin E_1; W=wash. Both drug concentrations are in μg/ml.

of tracheal muscle from the cat, monkey, guinea-pig, ferret and sheep, but had no effect on rabbit or pig tracheal muscle preparations in concentrations of up to 50 mg/ml.

Prostaglandin antagonized contractions produced by barium chloride in tracheal preparations of the cat, guinea-pig and ferret, and inhibited spontaneous activity in sheep tracheas. In five experiments on monkey tracheas, prostaglandin E_1 (0.25 to 5 μg/ml.) had no effect. Fig. 4 shows that prostaglandin E_1 (1.25 μg/ml.)

TABLE 1

THRESHOLD CONCENTRATIONS OF PROSTAGLANDIN E_1 CAUSING INHIBITION OF TONE IN ISOLATED TRACHEAL MUSCLE PREPARATIONS

Asterisks indicate that no inhibition could be demonstrated since the preparation had no initial tone even in the presence of the stimulant compound added. Values are μg/ml. of prostaglandin E_1 which inhibited tone in the presence of the stimulants indicated

	Threshold concentration (μg/ml.) of prostaglandin acting on				
	Contraction due to				
Species	Acetylcholine	Dihydroergotamine	Barium chloride	Histamine	Inherent tone
Cat	0·001	0·001	0·25	*	*
Monkey	0·02	*	>2·5	*	*
Rabbit	0·05	*	*	*	*
Guinea-pig	0·005	*	<0·25	0·005	0·005
Ferret	0·005	*	0·25	*	0·005
Sheep	3·0	*	0·05	*	>1·0
Pig	0·25	*	*	*	>2·0

relaxed a guinea-pig trachea which had been previously contracted by barium chloride (0.25 mg/ml.).

The effects of prostaglandin E_1 on isolated tracheal muscle preparations of six species are summarized in Table 1.

Resistance to inflation of the lung in vivo

In the rabbit, prostaglandin E_1 usually inhibited the rise in resistance to inflation caused by vagal stimulation. Fig. 5 illustrates an experiment where prostaglandin

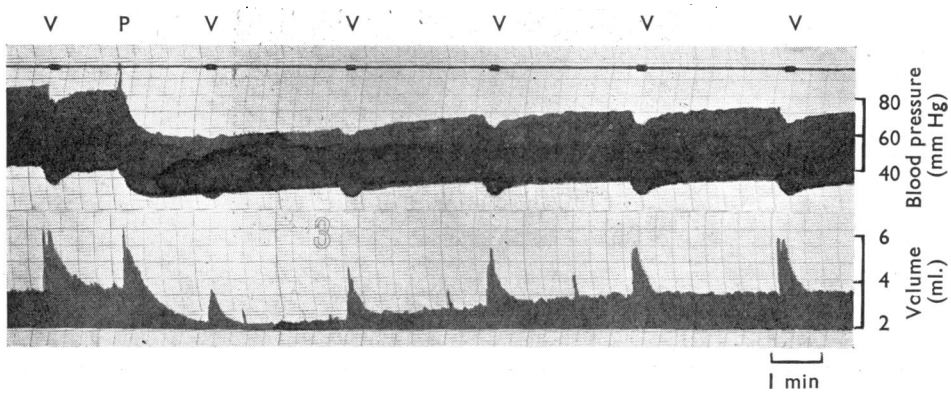

Fig. 5. Rabbit, 2.5 kg, anaesthetized with urethane. Uppermost trace, events marker; middle trace, arterial blood pressure; lowest trace, tidal overflow volume (see Methods). V=stimulation of the left vagus nerve for 10 sec; P=prostaglandin E_1 (4 μg) injected intravenously.

E_1 (1.6 μg/kg) lowered resistance to inflation and slightly inhibited the response to vagal stimulation. The threshold dose was about 0.4 μg/kg. In the guinea-pig, prostaglandin E_1 (0.1 to 0.25 μg/kg) antagonized the responses to vagal stimulation and to histamine (0.08 to 0.16 μg/kg) (Fig. 6). In the cat, prostaglandin E_1, in doses as low as 0.3 μg/kg, increased resistance to inflation and heart rate. Fig. 7 shows that prostaglandin E_1 (3.6 μg/kg) increased the resistance to inflation, which reached a maximum after the blood pressure had returned to the control level. The heart rate increased from 150 to 180 beats/min within 40 sec and returned to the control value in 7 min.

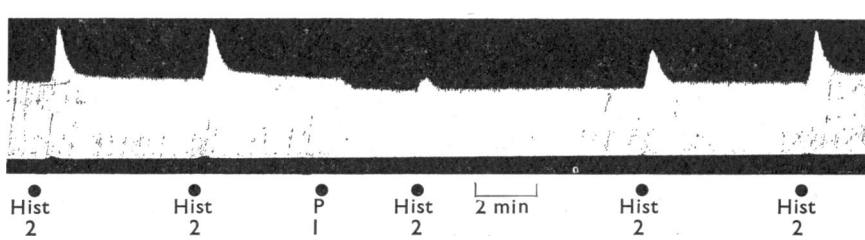

Fig. 6. Guinea-pig, 0.24 kg, anaesthetized with urethane. Record of tidal overflow volume (see Methods). Hist=histamine; P=prostaglandin E_1. All doses are in μg and were injected intravenously.

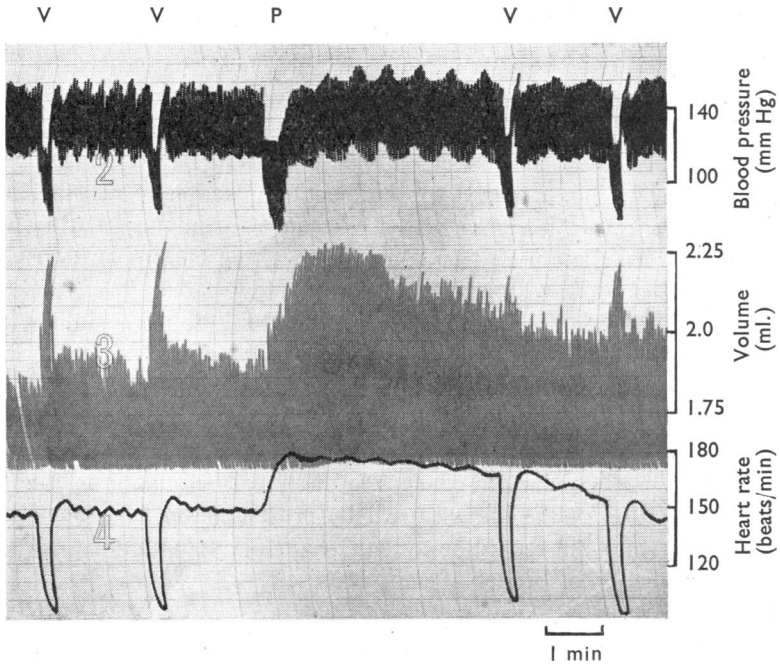

Fig. 7. Cat, 2.8 kg, anaesthetized with chloralose and urethane. Uppermost trace, arterial blood pressure; middle trace, tidal overflow volume (see Methods); lowest trace, heart rate. V= stimulation of left vagus nerve for 10 sec; P=prostaglandin E_1, 10 μg injected intravenously.

DISCUSSION

Although prostaglandin was originally discovered by its stimulant action on isolated smooth muscle preparations, prostaglandin E_1 has recently been shown to be a potent inhibitor *in vivo* of smooth muscle of the reproductive tract of the rabbit (Horton, Main & Thompson, 1963). The experiments reported above show that prostaglandins also relax respiratory smooth muscle. Horton & Main (1963) found that prostaglandins E_1, E_2 and E_3 decreased the tone of rabbit Fallopian tubes *in vivo,* whereas prostaglandin $F_{1\alpha}$ increased the tone. In contrast, all four prostaglandins have an inhibitory action on cat isolated tracheal preparations when these are first contracted by acetylcholine ; this preparation usually lacks tone. The two other known prostaglandins, $F_{2\alpha}$ and F_3 , were not tested. However, since prostaglandin $F_{2\alpha}$ has a range of biological activity similar to prostaglandin $F_{1\alpha}$ (Änggård & Bergström, 1963) it is possible that the property of relaxing tracheal muscle *in vitro* may be common to all prostaglandins.

The cat isolated tracheal preparation is 500-times more sensitive to prostaglandin E_1 than to $F_{1\alpha}$, a difference greater than with any other biological preparation so far tested. When the cat isolated trachea and rabbit jejunum preparations are used for the tests, the index of discrimination between prostaglandin E_1 and $F_{1\alpha}$ is approximately 1,000. These two preparations could therefore be used to distinguish between prostaglandin $F_{1\alpha}$ and prostaglandins of the E series in preference to the

guinea-pig ileum and rabbit jejunum preparations, previously suggested by Horton & Main (1963), for which the index of discrimination is 100.

The inhibitory action of prostaglandin E_1 on respiratory tract smooth muscle was also demonstrated *in vivo* in the rabbit and guinea-pig. In these experiments bronchoconstriction (increase in resistance to inflation of the lungs) induced by drugs or vagal stimulation was antagonized by intravenous injection of prostaglandin E_1 and there was sometimes a lowering of the resistance to inflation. Although low concentrations of prostaglandin E_1 relaxed cat tracheal muscle preparations *in vitro*, the intravenous injection of prostaglandin in doses as low as 0.3 μg/kg caused a rise in the resistance to inflation. Änggård & Bergström (1963) found a similar increase in " bronchial resistance " in the anaesthetized cat with prostaglandin $F_{2\alpha}$ in much higher doses (15 to 30 μg/kg). In their experiments prostaglandin F_2 caused bradycardia whereas in the present investigation prostaglandin E_1 caused a conspicuous tachycardia. The evidence put forward by Änggård & Bergström (1963) suggests that the effects may be secondary to changes in pulmonary circulation: the increase in resistance to inflation of the lungs caused by prostaglandin E_1 could be due to displacement of air into the recording system secondary to pulmonary congestion rather than to contraction of bronchial smooth muscle.

Linn, Shunk, Folkers, Ganley & Robinson (1961) found a smooth muscle stimulating factor " SRS-S " in an acidic lipid extract of normal swine lungs. Bergström and his co-workers have recently reported the presence of several prostaglandins, but predominantly $F_{2\alpha}$, in the lungs of all species so far investigated. A direct comparison between " SRS-S " and the prostaglandins using the isolated tracheal preparation might help to establish whether the activity of the " SRS-S " extract is due to a prostaglandin.

The discovery of prostaglandins in lung tissue raised the question of their possible physiological role in the respiratory tract. The present demonstration of inhibition of respiratory tract smooth muscle in several species suggests that prostaglandins may act to mediate inhibition in smooth muscle.

Prostaglandin E_2 is 500-times more potent than prostaglandin $F_{1\alpha}$ in relaxing cat tracheal muscle, and Änggård & Bergström (1963) have shown that prostaglandin $F_{2\alpha}$ is about 1- to 10-times more active than prostaglandin $F_{1\alpha}$. It is therefore probable that prostaglandin E_2 is 50- to 500-times more active than its corresponding alcohol prostaglandin F_2 and that, in spite of the higher concentration of prostaglandin $F_{2\alpha}$ in the lung, prostaglandin E_2 is of greater physiological importance.

I am very grateful to Professor S. Bergström for gifts of the pure prostaglandins.

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