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COMPARISON OF THE ACTIONS OF U-46619, A PROSTAGLANDIN H_2-ANALOGUE, WITH THOSE OF PROSTAGLANDIN H_2 AND THROMBOXANE A_2 ON SOME ISOLATED SMOOTH MUSCLE PREPARATIONS

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1 The actions of the prostaglandin H_2 (PGH_2) analogue, U-46619, have been compared with those of PGH_2 and thromboxane A_2 (TxA_2) on a range of isolated smooth muscle preparations in a superfusion cascade system.

2 U-46619 was a potent agonist on guinea-pig lung strip, dog saphenous vein and rat and rabbit aortae. In contrast, U-46619 was weak or inactive on guinea-pig ileum and fundic strip, cat trachea and dog and cat iris sphincter muscles, preparations on which either PGE_2 or $PGF_{2\alpha}$ was the most potent agonist studied.

3 PGH_2 was active on all of the preparations and displayed little selectivity. On some of the preparations, the actions of PGH_2 may have been mediated indirectly by conversion to other prostanoids.

4 In contrast, TxA_2 displayed the same pattern of selectivity as U-46619, being a potent agonist on the lung strip and vascular preparations but weak or inactive on the others.

5 It is suggested that U-46619 is a selective TxA_2-mimetic and that it should therefore be a valuable tool in the study of the actions of TxA_2.

Introduction

The prostaglandin endoperoxides, PGG_2 and PGH_2 (Hamberg & Samuelsson, 1973; Hamberg, Svensson, Wakabayashi & Samuelsson, 1974) and thromboxane A_2 (TxA_2) (Hamberg, Svensson & Samuelsson, 1975a), unlike other cyclo-oxygenase products, all potently contract isolated vascular smooth muscle and aggregate platelets. However, study of the biological actions of these substances is complicated by their chemical instability. In the case of the endoperoxides there is the additional complication that they can break down spontaneously or be converted enzymatically to other biologically active prostanoids and it is frequently not clear whether their actions are direct or indirect through conversion to these other substances (Moncada & Vane, 1979).

One approach to the study of the biological activity of such unstable substances is to use chemically stable analogues. A number of stable analogues of PGH_2 have been synthesized, and they resemble the endoperoxides and TxA_2 in that they aggregate platelets and contract isolated vascular smooth muscle (Malmsten, 1976). We have previously shown (Apperley, Coleman, Kennedy & Levy, 1979; Coleman, Humphrey, Kennedy, Levy & Lumley, 1980b) that an epoxymethano analogue of PGH_2, U-46619 (Bundy, 1975) is a highly potent agonist on guinea-pig lung strip, dog saphenous vein and rat and rabbit aortae; whereas on some other preparations, on which either PGE_2 or $PGF_{2\alpha}$ was a highly potent agonist, U-46619 was weak or inactive. It was therefore of interest to compare the actions of TxA_2 and PGH_2 with those of U-46619 on the same preparations and such a comparison is the subject of the present paper. A preliminary account of this work has been presented to the British Pharmacological Society (Coleman, Humphrey, Kennedy, Levy & Lumley, 1980a).

Methods

Isolated smooth muscle preparations

The following preparations were used: guinea-pig lung strip, prepared according to the method of Lulich, Mitchell & Sparrow (1976); dog saphenous vein strip (Humphrey, 1978); rabbit and rat aortic strips, prepared according to the method of Furchgott & Bhadrakom (1953); guinea-pig ileum (Horton & Main, 1973); guinea-pig fundic strip, prepared according to the method of Vane (1957); dog and cat iris sphincter muscles, prepared accord-

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ing to the method of van Alphen & Angel (1975); and cat tracheal strips, prepared according to the method of Coburn & Tomita (1973).

Preparations were mounted for cascade superfusion (Vane, 1964). This technique allowed the most efficient use of substances such as PGH_2 and TxA_2, which are laborious to prepare and are only available in small quantities, since the same sample could be tested simultaneously on up to four preparations. The preparations were suspended under a resting tension of 1 g (dog saphenous vein, guinea-pig ileum and fundus and cat trachea) or 0.5 g (guinea-pig lung strip, rat and rabbit aortae, cat and dog iris sphincter muscles) and changes in tension measured isometrically. The preparations were superfused at a rate of 10 ml/min with modified Krebs solution (Apperley, Humphrey & Levy, 1976) at 37°C containing indomethacin (1.4×10^{-6} mol/l), atropine (2×10^{-7} mol/l) and phenoxybenzamine (3.5×10^{-7} mol/l) and gassed with 5% CO_2 in oxygen.

In those experiments where cat trachea was used atropine was omitted, and acetylcholine (7×10^{-5} mol/l) was added to the fluid superfusing this preparation. Cat trachea was always placed at the bottom of the cascade so that acetylcholine did not superfuse the other preparations. Otherwise, preparations were arranged in random order to minimize any influence the position in the cascade might have on the activity of unstable substances such as PGH_2 and TxA_2. However, the time taken for the passage of the superfusion fluid from top to bottom of the cascade was 3–4 s which is short compared to the half-life of PGH_2 (5 min at 37°C; Hamberg *et al.*, 1974) and TxA_2 (30 s at 37°C; Svensson, Strandberg, Tuvemo & Hamberg, 1977) and no variations attributable to position in the cascade were seen.

Preparation of prostaglandin H_2 and thromboxane A_2

PGH_2, prepared by incubating arachidonic acid with ram seminal vesicle microsomes as described by Gorman, Sun, Miller & Johnson (1977), was stored at −70°C in anhydrous diethyl ether until required. For use, the diethyl ether was evaporated under a N_2 stream and the PGH_2 redissolved in Krebs solution at 0°C to give a concentration of 5 μg/ml. Doses of PGH_2 were taken as aliquots from this stock solution. Dosing was begun 15 s after addition of the Krebs solution and was always complete within a further 105 s. TxA_2 was prepared essentially as described by Moncada, Needleman, Bunting & Vane (1976); after removal of the diethyl ether, PGH_2 was redissolved (5 μg/ml) in a suspension of indomethacin-treated sheep platelet microsomes in 0.1 M Tris buffer (pH 7.5) at 0°C. Doses of TxA_2 were then obtained as described for PGH_2. Preliminary experiments showed that conversion of PGH_2 to thromboxane was complete within 15 s. Doses of TxA_2 were arbitrarily based on an assumed 100% conversion of PGH_2. The PGH_2 and TxA_2 prepared had half-lives similar to those expected (see above).

Design of experiments

Dose-effect curves for PGE_2, $PGF_{2\alpha}$, PGH_2, TxA_2 and U-46619 were constructed 'cumulatively', each dose being administered at, or as close as possible to, the time of the peak response to the preceding dose.

In those experiments where the effects of PGE_2, $PGF_{2\alpha}$ and U-46619 were compared, $PGF_{2\alpha}$ dose-effect curves were repeated until sensitivity was constant (2–3 times), and a further dose-effect curve for either PGE_2 or U-46619 was then obtained. Dose-effect curves for $PGF_{2\alpha}$ were again repeated until constant (2–3 times) and finally a dose-effect curve for the remaining prostanoid was obtained. The $PGF_{2\alpha}$ dose-effect curves obtained before the first and second test prostanoid were similar and in the interests of clarity, only that obtained before the second test prostanoid is shown in Figure 1.

In those experiments where the effects of PGH_2 and TxA_2 were compared, dose-effect curves for PGH_2 were repeated until sensitivity of the preparation was constant (2–3 times), and then a further dose-effect curve for PGH_2 incubated with platelet microsomes (to generate TxA_2) was obtained. Due to the limitations of the generating systems, the highest dose of PGH_2 and TxA_2 which could be tested was 1500 ng.

Drugs

The following drugs were used: acetylcholine chloride (BDH), arachidonic acid (Sigma), atropine sulphate (BDH), imidazole (Sigma), indomethacin (Merck, Sharp & Dohme), phenoxybenzamine hydrochloride (Smith Kline & French), prostaglandin E_2 (Ono), prostaglandin $F_{2\alpha}$ tromethamine salt (Upjohn), 11α, 9α-epoxymethano-prostaglandin H_2 (U-46619, synthesized by Dr A.H. Wadsworth, Chemistry Research Department, Glaxo Group Research, Ware Division).

Results

Effects of U-46619, prostaglandins E_2 and $F_{2\alpha}$

Since our previous studies (Apperley *et al.*, 1979; Coleman *et al.*, 1980b) were carried out on preparations suspended in organ baths, we first examined the effects of U-46619, PGE_2 and $PGF_{2\alpha}$ in cascade superfusion. U-46619 was a potent agonist

(threshold dose 10–100 ng) on guinea-pig lung strip, dog saphenous vein, and rat and rabbit aortae. On these preparations, U-46619 was between 100 and 1000 times more potent than PGE_2 and $PGF_{2\alpha}$ (see Figure 1).

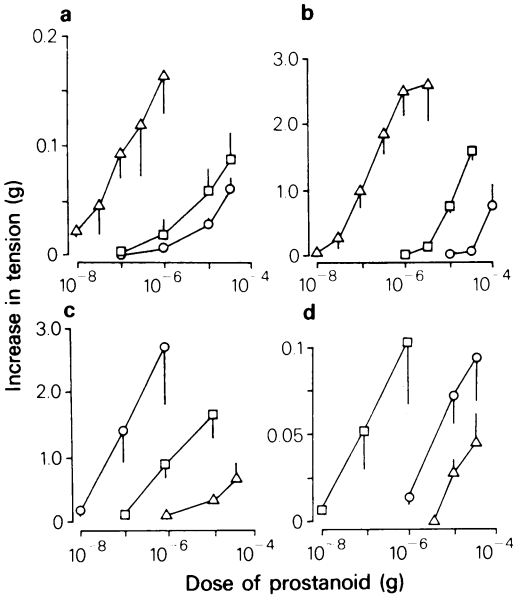

Figure 1 Dose-effect curves to U-46619 (△), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) (□) and PGE_2 (○) on guinea-pig lung (a), dog saphenous vein (b), guinea-pig ileum (c) and cat iris (d) in a superfusion cascade. The ordinate scale shows the increase in tension (g), and the abscissa scale dose of prostanoid (g). Each point is the mean of 3–5 determinations; vertical lines show s.e.mean.

In contrast, U-46619 was a much weaker agonist on the other preparations. On guinea-pig ileum and guinea-pig fundus, PGE_2 (threshold dose 1–10 ng) was the most potent of the three agonists in producing contraction, whilst $PGF_{2\alpha}$ was at least 10 times and U-46619 at least 100 times less potent than PGE_2 (Figure 1). PGE_2 also potently relaxed cat trachea, whilst $PGF_{2\alpha}$ and U-46619 were inactive in doses up to 10 μg. On cat and dog iris sphincter muscles, $PGF_{2\alpha}$ (threshold dose approximately 10 ng) was the most potent agonist, whilst PGE_2 was 10–100 times and U-46619 100–1000 times less potent than $PGF_{2\alpha}$ (Figure 1). These results are in good agreement with those we obtained previously using preparations suspended in organ baths (Apperley *et al.*, 1979; Coleman *et al.*, 1980b).

Effects of prostaglandin H_2 and thromboxane A_2

PGH_2 (15–1500 ng) caused dose-related contractions of all preparations except cat trachea, which it relaxed in a dose-related fashion. In contrast to the other prostanoids studied, PGH_2 displayed little selectivity, threshold doses ranging from 15–150 ng on all preparations. Representative recordings are shown in Figure 2.

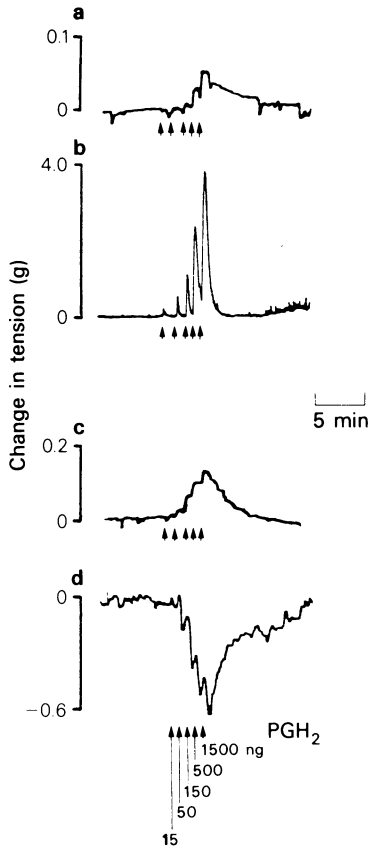

Figure 2 Effects of increasing doses (15–1500 ng) of prostaglandin H_2 (PGH_2) on rat aorta (a), guinea-pig ileum (b), dog iris (c), and cat trachea (d) in a superfusion cascade. PGH_2 caused dose-related contractions of rat aorta, guinea-pig ileum and dog iris, and relaxations of cat trachea. Changes in tension are shown in g. Elevated tone was induced in cat trachea by the addition of acetylcholine (7.0×10^{-5} M) to the fluid superfusing this tissue alone; the increase in resting tension produced by this concentration of acetylcholine was usually about 2–3 g.

On those preparations where U-46619 was a potent agonist, namely guinea-pig lung strip, dog saphenous vein, rabbit and rat aortae, TxA_2 was more potent than PGH_2. Although the threshold doses of PGH_2 and TxA_2 were similar on these preparations, dose-effect curves for TxA_2 were steeper than those for PGH_2 (see Figure 3). In con-

trast, on those preparations on which U-46619 was weak or inactive, TxA_2 was also weak or inactive.

On guinea-pig ileum, guinea-pig fundus and dog and cat iris sphincter muscles, the threshold doses of TxA_2 were much higher than those of PGH_2 and the dose-effect curve shallower (see Figure 3). TxA_2, like U-46619, neither contracted nor relaxed cat trachea.

The selective thromboxane synthetase inhibitor imidazole (Moncada, Bunting, Mullane, Thorogood, Vane, Raz & Needleman, 1977) was used to confirm that the biological activity produced by incubation of PGH_2 with platelet microsomes was due to TxA_2 formation. When PGH_2 was incubated with microsomes in the presence of imidazole (300 μg/ml), the biological activity of the mixture on all of the preparations was similar to that of PGH_2 alone (see Figure 3).

Discussion

The results of the present study confirm our previous findings (Apperley *et al.*, 1979; Coleman *et al.*, 1980b) that U-46619 is a potent agonist on guinea-pig lung strip, dog saphenous vein and rabbit and rat aortic strips whilst it is weak or inactive on guinea-pig ileum, guinea-pig fundic strip, cat trachea and cat and dog iris sphincter muscles. Furthermore, we have now shown that TxA_2 has the same profile of activity on these preparations, being a potent agonist on the lung strip and vascular preparations, but weak or inactive on the others. In contrast, however, PGH_2 displayed little selectivity. Thus, despite being chemically an analogue of PGH_2, U-46619 more closely resembles TxA_2 in its biological activity and may therefore be considered as a selective TxA_2 mimetic. Indeed, preliminary results with the receptor blocking drug AH19437 are consistent with the view that U-46619 and TxA_2 act at the same receptor (Coleman, Collington, Geisow, Hornby, Humphrey, Kennedy, Levy, Lumley, McCabe & Wallis, 1981; Coleman, Humphrey, Kennedy, Levy & Lumley, 1981). However, further work will be necessary to demonstrate this definitively, particularly since it has been suggested that there are distinct 'endoperoxide' and 'thromboxane' receptors (MacIntyre & Willis, 1978) and more than one type of thromboxane receptor (Fitzpatrick, Bundy, Gorman & Honohan, 1978).

In contrast to the other prostanoids used in the present study, PGH_2 displayed little selectivity, having similar potency on all preparations. Previous work (Hamberg & Samuelsson, 1973; Hamberg, Hedqvist, Strandberg, Svensson & Samuelsson, 1975b; Svensson *et al.*, 1977) has shown that whilst prostaglandin endoperoxides are more potent than PGE_2 and $PGF_{2\alpha}$ in contracting vascular smooth

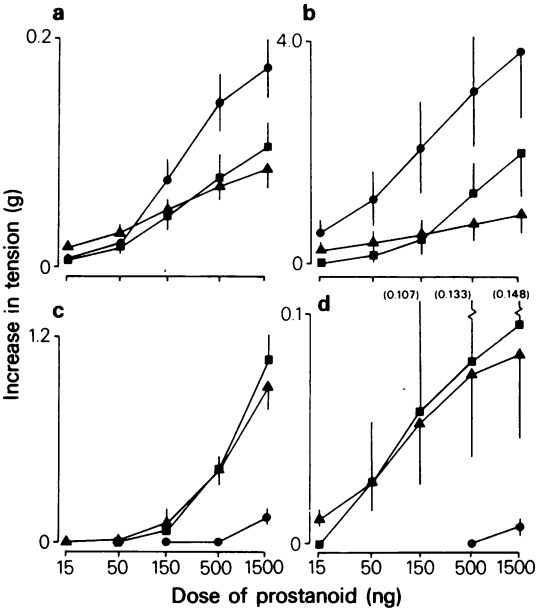

Figure 3 Effects of prostaglandin H_2 (PGH_2) and thromboxane A_2 (TxA_2) on guinea-pig lung (a), dog saphenous vein (b), guinea-pig ileum (c) and dog iris (d) in a superfusion cascade. Dose-effect curves to PGH_2 (▲), PGH_2 pre-incubated with sheep platelet microsomes (SPM) for 15–120 s to generate TxA_2 (●) and dose-effect curves to PGH_2 pre-incubated with SPM and imidazole (300 μg/ml) 15–120 s (■) are shown. The ordinate scale shows the increase in tension (g), and the abscissa scale the dose of PGH_2 (ng). Each point is the mean of 3–5 determinations; vertical lines show s.e.mean.

muscle, they are less potent in contracting gastro-intestinal smooth muscle. The results of the present study suggest that these differences are not due to differences in the absolute potency of the endoperoxides on vascular and gastro-intestinal smooth muscle; rather they arise because PGE_2 and $PGF_{2\alpha}$ are much weaker agonists on vascular smooth muscle than on gastro-intestinal smooth muscle (e.g. see Figure 1).

As already noted (see Introduction), it is frequently not clear whether the biological actions of the endoperoxides are direct or indirect. It is likely that the contractile action of endoperoxides on vascular smooth muscle is direct, since blood vessels convert endoperoxides predominantly into a vasodilator prostaglandin, namely PGI_2 (Dusting, Moncada & Vane, 1979; Moncada & Vane, 1979). Since guinea-pig lung readily produces TxA_2 (Coleman, Kennedy & Sheldrick, 1980c), it might have been anticipated that the contractile actions of PGH_2 on guinea-pig lung strip would be mediated by conversion to TxA_2. However, responses of this preparation to PGH_2 are

not blocked by the thromboxane synthetase inhibitor, imidazole (unpublished observation), and may therefore be mediated directly. Therefore, in view of the similarity between the profiles of activity of TxA_2 and U-46619, together with the close chemical similarity between PGH_2 and U-46619, it is tempting to speculate that PGH_2 has a direct action on preparations containing thromboxane receptors, but that its actions on those preparations containing receptors sensitive to PGE_2 and $PGF_{2\alpha}$ are mediated indirectly by conversion to other prostanoids. However, much further work will be necessary to test this hypothesis.

In conclusion, our results suggest that the PGH_2 analogue, U-46619, is a selective TxA_2 mimetic. If this is so, then U-46619 will be a valuable tool for the study of the biological actions of TxA_2 and in the characterization of thromboxane receptors.

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