ELSEVIER

European Journal of Pharmacology 278 (1995) 49–54

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Role of thromboxane A_2 in bradykinin-induced human isolated small bronchi contraction

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Received 3 October 1994; revised 6 February 1995; accepted 10 February 1995

Abstract

We previously demonstrated that the bradykinin-induced contraction of human isolated small bronchi is inhibited by indomethacin, capsaicin (*N*-methyl-*N*-6-nonenamide) and ruthenium red but not by tachykinin receptor antagonists. The thromboxane A_2 receptor (TP receptor) antagonist GR32191 ((1*R*-(1α(*Z*),2β,3β,5α))-(+)-7-(5-(((1,1'-biphenyl)-4-yl)-methoxy)-3-hydroxy-2-(1-piperidinyl)cyclopentyl)-4-heptenoic acid, hydrochloride) (10^{-10} to 10^{-8} M) dose dependently inhibited the effect of bradykinin, suggesting the mediation of the TP receptor in the action of bradykinin. With higher concentrations of GR32191 (10^{-7} and 10^{-6} M) bradykinin induced a relaxation which was inhibited by indomethacin and by the bradykinin B_2 receptor antagonist Hoe 140 (D-Arg^0[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin). The thromboxane A_2 synthase inhibitor dazoxiben (4-(-2-(1*H*-imidazol-1-yl)ethoxy) benzoic acid hydrochloride) 10^{-6} M inhibited the bradykinin-induced contraction, suggesting that thromboxane A_2 was involved in TP receptor stimulation. The thromboxane A_2 mimetic U-46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$)-induced contraction of human distal bronchi was not inhibited by capsaicin and ruthenium red. Our data suggest that bradykinin contracts human isolated small bronchi through thromboxane A_2 release. The inhibitory effect of ruthenium red and capsaicin on the bradykinin response may be due to inhibition of thromboxane A_2 release or arachidonic mobilisation.

Keywords: Bradykinin; Capsaicin; Thromboxane A_2; Smooth muscle, airway

1. Introduction

Bradykinin is a naturally occurring inflammatory nonapeptide generated by the cleavage of kininogen (Regoli and Barabé, 1980). On the basis of its mediator properties, it has been suggested that bradykinin may be involved in the pathophysiology of asthma (Barnes, 1992). Asthmatic patients have elevated kinin concentrations in plasma (Abe et al., 1967) and in bronchoalveolar lavage fluid after antigen challenge (Christiansen et al., 1987). Inhaled bradykinin causes bronchoconstriction in asthmatics but only chest discomfort in normal subjects (Fuller et al., 1987). We have previously demonstrated that bradykinin is a potent constrictor of human isolated small (diameter 0.5–1 mm), but not large, bronchi and that this effect involves a cyclooxygenase product since the contractile response to bradykinin was abolished by indomethacin (Molimard et al., 1994).

Since bradykinin-induced airway obstruction has been shown to be mediated, at least partly, via thromboxane A_2 in guinea-pig (Arakawa et al., 1992; Kawikova et al., 1993), and since the stable thromboxane A_2 mimetic U-46619 contracts human large (inner diameter 1–4 mm) airway smooth muscle in vitro (Armour et al., 1989; Coleman and Sheldrick 1989; Lumley et al., 1989; Featherstone et al., 1990), the aim of this study was to investigate the possible role of thromboxane A_2 in the bradykinin-induced contraction of smooth muscle of human distal airways. Therefore, we studied the effect of GR32191, a potent thromboxane A_2 receptor (TP receptor) antagonist, and of da-

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Elsevier Science B.V.
SSDI 0014-2999(95)00103-4

zoxiben, a potent thromboxane A_2 synthase inhibitor, on contractions of human distal airways induced by bradykinin (Lumley et al., 1989; Stenton et al., 1992), and compared the effects of bradykinin and U-46619 on human airways. Since we previously demonstrated that bradykinin-induced contractions are inhibited by capsaicin and ruthenium red in human small airways (Molimard et al., 1994), we compared the inhibitory effects of these products on U-46619- and bradykinin-induced contractions of human airway smooth muscle.

2. Materials and methods

2.1. Human isolated bronchi preparation

Bronchial tissues were removed from patients (mean age, 61.5 years; range 43–78 years) with lung cancer at the time of the surgical operation. All were male and previous smokers. None was asthmatic. Just after resection, segments of bronchi with an inner diameter of 3–5 mm (large bronchi) or 0.5–1 mm (small bronchi) were taken from an area as far as possible from the malignancy. They were placed in oxygenated Krebs-Henseleit solution (NaCl, 119; KCl, 5.4; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 11.7 mM) and stored overnight at 4° C. After removal of adhering fat and connective tissues, 4–8 rings of the same bronchus were prepared. Each set of bronchial rings was suspended under an initial tension of 2.5 g (large bronchi) or 1.5 g (small bronchi) in Krebs-Henseleit solution, bubbled with 95% O_2-5% CO_2 and maintained at 37° C. Changes in force of contraction were measured isometrically with a strain gauge and amplifiers and displayed on an I.O.S.-Moise 3 recorder (EMKA, Mitry Mory, France).

Each experiment began by contracting the bronchial strips to maximal tension with acetylcholine (1 mM), and then maximal relaxation was induced with theophylline (3 mM). During the next 90 min, the tissues were washed every 15 min. Experiments were conducted on parallel groups of 4–8 rings, one ring serving as control.

2.2. Protocol

Concentration-response curves for bradykinin (10^{-9} to 10^{-5} M) or for U-46619 (10^{-10} to 10^{-6} M) were recorded by applying increasing concentrations of drugs, at 3- to 6-min and 10- to 20-min intervals, respectively, in logarithmic increments.

Pretreatments with GR32191 (10^{-10} to 10^{-6} M), ruthenium red 10^{-5} M and capsaicin 10^{-5} M were given 1 h before cumulative concentration-response curves for bradykinin or U-46619 were made. Pretreatments with Hoe 140 10^{-6} M and dazoxiben 10^{-6} M were given 15 min before concentration-response curves for bradykinin were made.

Concentrations and pretreatment duration were chosen according to Lumley et al. (1989) for GR32191 and U-46619, to Brownlie et al. (1993) for dazoxiben and to our previous studies for other drugs (Molimard and Advenier, 1993; Molimard et al., 1994).

Responses are expressed as percentages of the maximal contraction induced by acetylcholine (1 mM) or as percentages of the maximal relaxation induced by theophylline (3 mM).

2.3. Statistical analysis

The agonist potency was defined as pD_2, i.e. the negative log of the drug concentration that caused 50% of the maximal effect induced by drug ($pD_2 = -\log EC_{50}$). The maximal effect (E_{max}) is expressed as a percentage of the maximal contraction induced by acetylcholine (1 mM). pA_2 values were determined according to Arunlakshana and Schild (1959). Statistical analysis of the results was performed using variance analysis and Student's *t*-test. All values in the text and figures are expressed as means ± standard error of the mean (S.E.M). *P* values lower than 0.05 were considered to be significant.

2.4. Drugs

The drugs used were: bradykinin, capsaicin (*N*-methyl-*N*-6-nonenamide), ruthenium red, U-46619 (9,11-dideoxy-11α,9α-epoxymethano-prostaglandin $F_{2\alpha}$), indomethacin (Sigma, St. Louis, MO, USA), GR32191 ((1*R*-(1α(*Z*),2β,3β,5α))-(+)-7-(5-(((1,1'-biphenyl)-4-yl)-methoxy)-3-hydroxy-2-(1-piperidinyl)cyclopentyl)-4-heptenoic acid, hydrochloride) (kind gift of Dr Coleman, Glaxo, Greenford, UK), dazoxiben (4-(-2-(1*H*-imidazol-1-yl)ethoxy) benzoic acid hydrochloride) (kind gift of Dr Bostock, Pfizer, Sandwich, UK), Hoe 140 (D-Arg^0[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin, Hoechst, Puteaux, France), acetylcholine (PCH, Paris, France). Theophylline sodium anisate was used as proprietary injectable solution (Delalande, Paris, France). U-46619 was dissolved in distilled water at a concentration of 10 μM and kept in small aliquots at −20° C until used. A fresh aliquot was used for each experiment. All drugs were dissolved in distilled water and then diluted in Krebs solution, except for capsaicin and indomethacin, which were dissolved in ethanol, then diluted in Krebs solution. The final concentration of ethanol (0.03%) did not alter the effect of acetylcholine, bradykinin and U-46619.

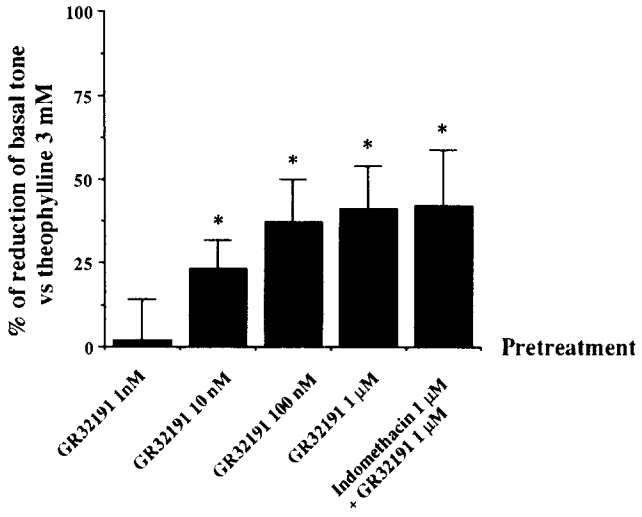

Fig. 1. Effects of GR32191 (10^{-9} to 10^{-6} M) and of indomethacin 10^{-6} M plus GR32191 10^{-6} M on resting tone of small airways. Values are means ± S.E.M. Significant relaxations from baseline are expressed by: * $P < 0.05$ ($n = 7$).

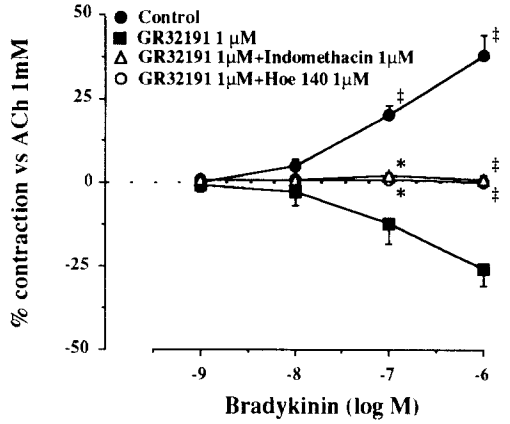

Fig. 3. Effects of indomethacin 10^{-6} M and Hoe 140 10^{-6} M on bradykinin-induced relaxation after GR32191 10^{-6} M pretreatment. Values are means ± S.E.M. Significant differences from bradykinin-induced relaxation after GR32191 10^{-6} M pretreatment are expressed by: * $P < 0.05$, ‡ $P < 0.001$ ($n = 4$).

3. Results

3.1. Effects of GR32191 on bradykinin concentration-response curves

Bradykinin induced a transient contraction in all the 11 small bronchi studied with a pD_2 of 7.0 ± 0.3 and an E_{max} of 33.1 ± 3.2% of the contraction elicited by acetylcholine 1 mM. The contraction reached its maximum within 6 min as described earlier (Molimard et al., 1994).

The TP receptor antagonist GR32191 dose dependently decreased the resting tone of both large (data not shown) and small bronchi (Fig. 1) but did not affect the acetylcholine 10^{-3} M-induced contraction. Fig. 2 shows that GR32191 dose dependently reduced (10^{-10} and 10^{-9} M) and abolished (10^{-8} M) the contractile effect of bradykinin. In the presence of higher concentrations of GR32191 (10^{-7} and 10^{-6} M) bradykinin induced a relaxation. Pretreatment with indomethacin 10^{-6} M or Hoe 140 10^{-6} M, which we previously have shown to inhibit bradykinin-induced contractions without inducing relaxation (Molimard et al., 1994), did not affect the resting tone but abolished the bradykinin-induced relaxation obtained after GR32191 10^{-6} M pretreatment. (Fig. 3).

3.2. Effects of dazoxiben on bradykinin concentration-response curves

Dazoxiben 10^{-6} M significantly shifted to the left the bradykinin-induced concentration-response curves ($n = 4$). A significant contractile effect of bradykinin was still observed at 10^{-7} M and 10^{-6} M (Fig. 4).

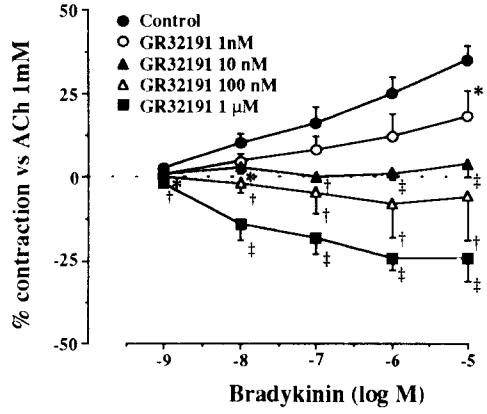

Fig. 2. Effects of GR32191 (10^{-9} to 10^{-6} M) on bradykinin (10^{-9} to 10^{-6} M) concentration-response curves. Values are means ± S.E.M. Significant differences from control are expressed by: * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ ($n = 7$).

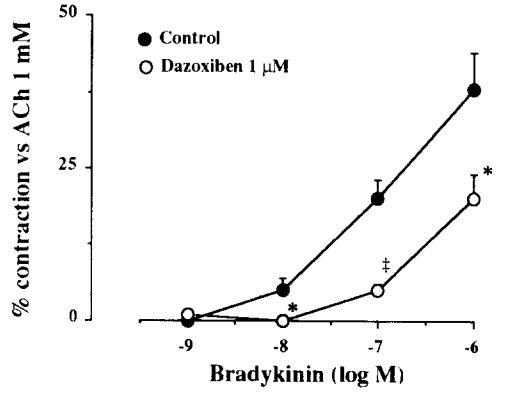

Fig. 4. Effects of dazoxiben 10^{-6} M on bradykinin (10^{-9} to 10^{-6} M) concentration-response curves. Values are means ± S.E.M. Significant differences from control are expressed by: * $P < 0.05$, ‡ $P < 0.001$ ($n = 4$).

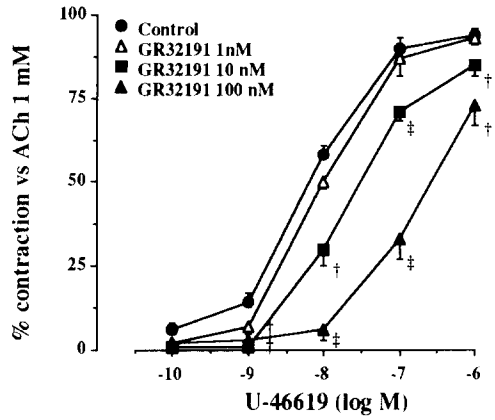

Fig. 5. Effects of GR32191 (10^{-9} to 10^{-7} M) on U-46619 concentration-response curves (10^{-10} to 10^{-6} M) for small isolated bronchi. Values are means ± S.E.M. Significant differences from control are expressed by: * $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$ ($n = 7$).

3.3. Effects of U-46619 on human bronchi

U-46619 potently contracted small ($n = 7$) and large ($n = 4$) airway smooth muscle. U-46619 was significantly more potent on small than on large airway smooth muscle, with a pD_2 of 8.35 ± 0.07 and 7.86 ± 0.05 respectively ($P < 0.001$). Furthermore, U-46619 had a significantly greater efficacy on small than on large airway smooth muscle, with an E_{max} of 92 ± 2% and 82 ± 3% respectively ($P < 0.05$).

GR32191 dose dependently and competitively inhibited U-46619-induced contractions of small airways with a pA_2 of 8.32 ± 0.13 and a slope of the Schild plot curve of 1.09 ± 0.09 ($n = 7$) (Fig. 5).

3.4. Effects of capsaicin and ruthenium red on U-46619-induced contractions

According to our previous studies, capsaicin 10^{-5} M and ruthenium red 10^{-5} M had no significant effect on the resting tone of human small airway smooth muscle (Molimard et al., 1994). Neither capsaicin 10^{-5} M nor ruthenium red 10^{-5} M had any effect on the U-46619 concentration-response curves (Fig. 6), contrary to what we observed for the bradykinin concentration-response curves (Molimard et al., 1994).

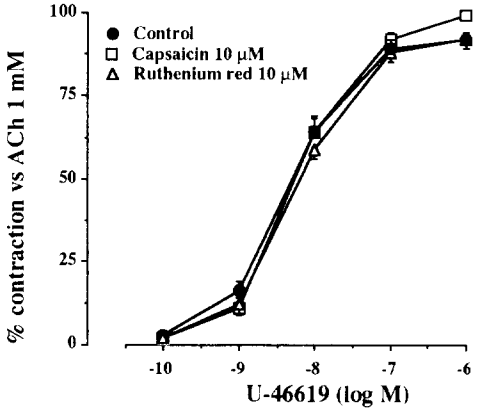

Fig. 6. Effects of capsaicin 10^{-5} M and ruthenium red 10^{-5} M on bradykinin (10^{-9} to 10^{-6} M) concentration-response curves ($n = 4$). Values are means ± S.E.M.

4. Discussion

Our results confirmed that bradykinin is a potent constrictor of human small airway bronchi. We demonstrated that GR32191, a potent and specific TP receptor inhibitor (Lumley et al., 1989; Armstrong et al., 1993), can completely suppress the contractile component of bradykinin action. Human airway TP receptors can be stimulated by thromboxane A_2 and by other prostanoids, such as endoperoxides, prostaglandins D_2 and $F_{2\alpha}$ (Featherstone et al., 1990). Thus, inhibition of bradykinin-induced contraction by TP receptor blockade does not necessarily imply that thromboxane A_2 is involved in the bradykinin effect. We therefore studied the effect of dazoxiben, a specific and potent (IC_{50} of 0.09 ± 0.01 μM in human platelet) thromboxane A_2 synthase inhibitor, on bradykinin-induced contractions (Brownlie et al., 1993). Dazoxiben strongly inhibited the contractile effects of bradykinin. The residual contractile effect observed with bradykinin 10^{-6} M after dazoxiben 10^{-6} M pretreatment may be due to the accumulation of endoperoxides or other prostanoids whose synthesis is induced by thromboxane A_2 synthase inhibition, as shown in human platelets (Brownlie et al., 1993), and subsequent TP receptor stimulation (Featherstone et al., 1990). Taken together, our results suggest that the bradykinin-induced contraction is at least partly mediated by thromboxane A_2.

With the highest concentration of GR32191 studied (10^{-6} M) bradykinin became relaxant. The relaxant effect was observed although GR32191 itself reduced the resting tone. This relaxation is also mediated by prostaglandins since it was abolished by the cyclooxygenase inhibitor indomethacin. Thus, both contractile and relaxant components of the bradykinin effect are mediated by the cyclooxygenase pathway, as we previously suspected from the demonstration that indomethacin completely abolished the bradykinin effect. We suggest that both types of prostanoids released are induced by bradykinin B_2 receptor stimulation, since we have previously shown that Hoe 140, a potent and specific bradykinin B_2 receptor antagonist (Hock et al., 1991), completely abolished the contractile effect of bradykinin (Molimard et al., 1994), and since we demonstrated in this study that Hoe 140 inhibits the bradykinin-induced relaxation observed after GR32191 pretreatment. Thus, we demonstrated that bradykinin stimulates the bradykinin B_2 receptor that induces the

release of cyclooxygenase products which contract (through TP receptor) or relax small airway smooth muscle.

We have previously shown that bradykinin-induced contractions are inhibited by capsaicin or ruthenium red pretreatment. Inhibition of tachykinin release seems not to be involved in this effect since neither NK_1 nor NK_2 receptor antagonist pretreatment ((±)-CP 96,345 and SR 48968 respectively) inhibited bradykinin-induced contractions (Molimard et al., 1994). It can be asked at which step of the bradykinin effect do ruthenium red and capsaicin interact? We therefore used the thromboxane A_2 stable mimetic U-46619, which has been shown to be a potent constrictor of human bronchi (1–4 mm inner diameter) through TP receptors since its effect is competitively antagonised by GR32191 (Armour et al., 1989; Coleman and Sheldrick, 1989; Lumley et al., 1989; Featherstone et al., 1990). With small bronchi (diameter 0.5–1 mm) our results showed that U-46619 is also a potent constrictor, even more potent and efficient than on large bronchi and its effect is competitively antagonised by GR32191. We demonstrated that U-46619-induced contractions are not antagonised by ruthenium red or capsaicin in concentrations that inhibit the effects of bradykinin. Thus, the antagonism exerted by capsaicin and ruthenium red on bradykinin-induced contractions is prior to TP receptor stimulation. Since capsaicin and ruthenium red at the highest concentration studied completely abolished bradykinin-induced contraction and relaxation in a non-competitive manner (Molimard et al., 1994), we suggest that capsaicin and ruthenium inhibit bradykinin-induced contraction before the prostanoid synthesis step induced by bradykinin B_2 receptor stimulation. Bradykinin-induced prostanoid release has been described by many authors in other models. By using specific inhibitors of phospholipase A_2, Juan (1977, 1979) suggested that bradykinin activates a calcium-dependent phospholipase A_2 in the isolated perfused ear of the rabbit, leading to the release of prostaglandins. In guinea-pig aorta smooth cells Zhang et al. (1991) have shown that bradykinin acts specifically on the cyclooxygenase step of the arachidonic acid cascade, since bradykinin enhanced the release of prostanoids but had no effect on the concentrations of free arachidonic acid in the presence or absence of indomethacin. The production of prostaglandins is primarily controlled by arachidonic acid release, and experiments with phospholipase A_2 inhibitors do not necessarily identify the point at which an agent intervenes in the cascade because the absence of free arachidonic acid eliminates all the subsequent metabolic steps with this substrate. It remains unclear how capsaicin and ruthenium red inhibit bradykinin B_2 receptor stimulation-induced prostanoid release. Is it through direct enzymatic (phospholipase A_2 or cyclooxygenase?) inhibition or through inhibition of intracellular pathways leading to activation of this enzyme? Could ruthenium red inhibit a calcium-dependent phospholipase A_2 through its ability to block calcium channels (Maggi et al., 1989)? Capsaicin has been reported to display a moderate activity against prostaglandin E_2 synthesis in intact human leukocytes (IC_{50} 73 μM) (Flynn et al., 1986) and to induce prostaglandin E_2 release in isolated perfused rabbit ear, but the possible role for substance P in this effect has not been addressed (Juan et al., 1980). We cannot answer these questions on the basis of our results, and thus the mechanism of the effect of capsaicin and ruthenium red on our preparation remains to be explored.

The relaxant effect of GR32191 pretreatment may be interpreted as a change in prostanoid balance responsible for basal tone. Interestingly, indomethacin had no significant effect on basal tone after GR32191 10^{-6} M pretreatment, although the relaxation was not maximal, suggesting a small role for relaxant prostaglandin on basal tone. Thus, beside the role of cysteinyl-leukotrienes in mediating intrinsic tone in isolated human airways (Ellis and Undem, 1993), the role of prostanoids on basal tone appears to be mainly contraction through TP receptor stimulation.

The segmental differences we previously observed in bradykinin action (Molimard et al., 1994) were not due to segmental differences in TP receptor distribution, since U-46619 contracted small airways and was more potent and effective on small than on large airways. Furthermore GR32191 reduced basal tone in large and small airways, suggesting that both types of airways are able to synthesise contractile prostanoids. Thus, differences in the distribution of bradykinin B_2 receptors, as shown by autoradiographic visualisation of bradykinin receptors in human lung (Mak and Barnes, 1991), are the most likely explanation of the lack of contractile effects of bradykinin on large airways.

To conclude, we have shown that bradykinin B_2 receptor stimulation induces the release of both contractile and relaxant prostanoids which entirely mediate the action of bradykinin on human isolated small airways. The contractile component of the action of bradykinin is due to TP receptor stimulation mainly by thromboxane A_2. Capsaicin and ruthenium red inhibit bradykinin-induced contraction through inhibition of the bradykinin-induced synthesis of prostanoids.

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