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Role of Ca^{2+} mobilization and Ca^{2+} sensitization in 8-iso-PGF_{2 α} -induced contraction in airway smooth muscle

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Clinical and Experimental Allergy

Summary

Background Isoprostanes are prostaglandin (PG)-like compounds synthesized by oxidative stress, not by cyclooxygenase, and increase in bronchoalveolar lavage fluid of patients with asthma. The airway inflammation implicated in this disease may be amplified by oxidants. Although isoprostanes are useful biomarkers for oxidative stress, the action of these agents on airways has not been fully elucidated.

Objective This study was designed to determine the intracellular mechanisms underlying the effects of oxidative stress on airway smooth muscle, focused on Ca^{2+} signalling pathways involved in the effect of 8-iso-PGF_{2 α} .

Methods Using simultaneous recording of isometric tension and F_{340}/F_{380} (an indicator of intracellular concentrations of Ca^{2+} , $[\text{Ca}^{2+}]_i$), we examined the correlation between tension and $[\text{Ca}^{2+}]_i$ in response to 8-iso-PGF_{2 α} in the fura-2 loaded tracheal smooth muscle.

Results Augmented tension and F_{340}/F_{380} by 8-iso-PGF_{2 α} were attenuated by ICI-192605, an antagonist of thromboxane A₂ receptors (TP receptors). Moreover, D609, an antagonist of phosphatidylcholine-specific phospholipase C, markedly reduced both the tension and F_{340}/F_{380} induced by 8-iso-PGF_{2 α} , whereas U73122, an antagonist of phosphatidylinositol-specific phospholipase C, modestly inhibited them by 8-iso-PGF_{2 α} . SKF96365, a non-selective antagonist of Ca^{2+} channels, markedly reduced both tension and F_{340}/F_{380} by 8-iso-PGF_{2 α} . However, diltiazem and verapamil, voltage-dependent Ca^{2+} channel inhibitors, modestly attenuated tension although their reduction of F_{340}/F_{380} was not different from that by SKF96365. Y-27632, an inhibitor of Rho-kinase, significantly attenuated contraction induced by 8-iso-PGF_{2 α} without reducing F_{340}/F_{380} , whereas GF109203X and Go6983, protein kinase C inhibitors, did not markedly antagonize them although reducing F_{340}/F_{380} with a potency similar to Y-27632.

Conclusion 8-iso-PGF_{2 α} causes airway smooth muscle contraction via activation of TP receptors. Ca^{2+} mobilization by SKF96365- and D609-sensitive Ca^{2+} influx and Ca^{2+} sensitization by Rho-kinase contribute to the intracellular mechanisms underlying the action of 8-iso-PGF_{2 α} . Rho-kinase may be a therapeutic target for the physiologic abnormalities induced by oxidative stress in airways.

Keywords isoprostane, oxidative stress, phospholipase C, Rho-kinase, thromboxane A₂ receptor

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Introduction

Isoprostanes are prostaglandin (PG)-like compounds generated non-enzymatically by peroxidation of membrane phospholipids by free radicals and reactive oxygen species [1]. These compounds are recognized as useful biomarkers for oxidative stress *in vivo*. Isoprostanes are increased in the serum, urine, exhaled breath condensate and bronchoal-

veolar lavage fluid of chronic inflammatory diseases of the respiratory system such as asthma and chronic obstructive pulmonary disease (COPD) [2–4]. On the other hand, isoprostanes are known to be capable of PG-like physiological effects. The airway inflammation implicated in asthma and COPD is further amplified by oxidative stress. Moreover, isoprostanes, which are synthesized by oxidative stress, are also involved in the pathophysiology of these diseases [5].

8-iso-PGF_{2α}, an isomer of PGF_{2α} has contractile effects via binding to thromboxane A₂ receptors (TP receptor) in airway smooth muscle [6, 7]. Moreover, this agent causes an increase in lung resistance *in vivo* [8]. These results indicate that airflow limitation in asthma and COPD may be mediated by an increase in the level of 8-iso-PGF_{2α} via oxidant stress, although there is substantial evidence that other mediators may play the major role. The intracellular mechanism of 8-iso-PGF_{2α} should be examined to clarify the pathophysiology induced by oxidants. However, little is currently known about the post-receptor processes associated with TP receptors in the action of 8-iso-PGF_{2α} on airway smooth muscle. In vascular smooth muscle, Ca²⁺ signalling pathways contribute to the action of 8-iso-PGF_{2α} via two mechanisms, i.e. (1) Ca²⁺ dynamics (mobilization) via both L-type voltage-dependent Ca²⁺ (VDC) channels and voltage-independent non-selective cation channels and (2) Ca²⁺ sensitization via Rho-kinase, a target protein of Rho (small G protein) [9–12]. Ca²⁺ signalling pathways as intracellular mechanisms underlying contraction induced by 8-iso-PGF_{2α} still remain controversial. Moreover, the correlation between the post-receptor processes and Ca²⁺ signalling pathways also remains poorly understood.

As described above, although oxidative stress may be an important pathogenesis for amplification of the inflammatory response in asthma and COPD, the no successful therapy for anti-oxidants has yet been developed. Therefore, for the development of a treatment against oxidative stress, it is necessary to investigate in detail the intracellular mechanisms underlying the action of isoprostanes on airway smooth muscle. Ca²⁺ mobilization and Ca²⁺ sensitization are essential factors as intracellular signal transduction pathways for the regulation of airway smooth muscle function.

The present study was designed to determine the post-receptor signal transduction processes underlying airway smooth muscle contraction induced by oxidative stress. Using fura-2 loaded intact tissues of guinea-pig trachea, we examined the effects of 8-iso-PGF_{2α} on airway smooth muscle in detail, focused on the post-receptor pathways involving Ca²⁺ mobilization and Ca²⁺ sensitization.

Materials and methods

All animal procedures were approved by the Animal Care and Use Committee of the Nagoya University Graduate School of Medicine.

Tissue preparation and solution

Male guinea pigs (300–350 g) were killed by injecting an overdose of anesthetics (150 mg/kg pentobarbital, i.p.) and tracheas were excised. The tracheal rings were opened

by cutting longitudinally at the cartilaginous region, and the epithelium was carefully dissected out. The normal bathing solution was composed of (in mM): NaCl 137, KHCO₃ 5.9, CaCl₂ 2.4, MgCl₂ 1.2, and glucose 11.8, bubbled with a mixture of 99% O₂ and 1% CO₂ (pH 7.4). The bathing solution was filled in the organ bath at a constant flow of 3 mL/min, and the resting tone was abolished by addition of indomethacin (2 μM) throughout the experiments.

Isometric tension recording and measurement of fura-2 fluorescence

The methods are essentially similar to those described previously [13, 14]. Muscle strips were prepared and treated with 10 μM of fura-2/AM for 4 h at room temperature (22–24 °C). The non-cytotoxic detergent, pluronic F-127 (0.01% w/v), was added to increase the solubility of fura-2/AM. After the loading, the chamber was filled with the normal bathing solution to wash out. Isometric tension and the fura-2 fluorescence of muscle strips were measured simultaneously, using a displacement transducer and a spectrofluorometer (CAF-110, Japan Spectroscopic, Tokyo, Japan). The intensities of fluorescence due to excitation at 340 (F₃₄₀) and 380 (F₃₈₀) nm were measured after background subtraction. The absolute amount of [Ca²⁺]_i was not calculated, because the dissociation constant of fura-2 for Ca²⁺ in smooth muscle cytoplasm is known to be different from that obtained *in vitro* [15]. The ratio of F₃₄₀ to F₃₈₀ (F₃₄₀/F₃₈₀) was used as a relative indicator of [Ca²⁺]_i. Methacholine (MCh; 1 μM) was applied for 5 min at every 15 min to establish a stable response and then the experiments were started. The experiments were carried out at 37 °C.

Experimental protocol

To examine the relationship between contraction and [Ca²⁺]_i in the action of isoprostanes, 8-iso-PGF_{2α} was cumulatively applied to the tissue. To examine the involvement of TP receptors in the action of 8-iso-PGF_{2α}, ICI-192605 (an antagonist of TP receptors) was applied to the tissues pre-contracted by 3 μM 8-iso-PGF_{2α}. To determine the mechanism of Ca²⁺ mobilization in the action of 8-iso-PGF_{2α}, SKF96365 (a non-selective inhibitor of Ca²⁺ channels), diltiazem and verapamil (inhibitors of VDC channels) were applied in the same way. To determine whether Ca²⁺ influx is involved in contraction by 8-iso-PGF_{2α}, this agent was applied in the presence of nominally free Ca²⁺ solution with 1 mM EGTA. On the other hand, to determine the involvement of Ca²⁺ sensitization in the action of 8-iso-PGF_{2α}, we applied GF109203X, Go6983 (inhibitors of protein kinase C, PKC), and Y-27632 (an inhibitor of Rho-kinase) to the tissues pre-contracted by 3 μM 8-iso-PGF_{2α}. Moreover, the intracellular response to

phospholipase C (PLC) in the action of 8-iso-PGF_{2 α} was determined by application of U73122 (an inhibitor of phosphatidylinositol-specific PLC, PI-PLC), and D609 (an inhibitor of phosphatidylcholine-specific PLC, PC-PLC). To determine whether the action of D609 is indeed specific, D609 was applied to the tissues pre-contracted not only by 8-iso-PGF_{2 α} but also by 60 mM K⁺, MCh, and U46619, a TP receptor analog. Tension and F_{340}/F_{380} in the resting state were taken as 0%, and contraction and F_{340}/F_{380} in response to these agents were expressed by taking 1 μM MCh-induced or 3 μM 8-iso-PGF_{2 α} -induced contraction as 100% during each experiment.

Materials

8-iso-PGF_{2 α} , PGF_{2 α} , and U46619 were obtained from Cayman Chemical (Ann Arbor, MI, USA). ICI-192605, MCh, indomethacin, SKF96365, verapamil, diltiazem, U73122, D609, GF109203X, Go6983, and pluronic F-127 were obtained from Sigma Chemical (St Louis, MO, USA). Y-27632 was obtained from Calbiochem (La Jolla, CA, USA). Fura-2/AM was from Dojin Laboratories (Kumamoto, Japan).

Statistical analysis

All data were expressed as means $\pm$ standard deviation. The concentrations of relaxant or contractile agents that produce 50% inhibition (IC₅₀) or 50% contraction (EC₅₀), respectively, were determined using non-linear regression analysis of each concentration-response curve. The statistical significance of all data was assessed by Student's *t*-test or repeated measures ANOVA with the Bonferroni *post hoc* test. A probability below 0.05 ($P < 0.05$) was considered to constitute a significant difference.

Results

Effects of 8-iso-PGF_{2 α} on tension and $[\text{Ca}^{2+}]_i$

Cumulative applications of 8-iso-PGF_{2 α} (0.1–30 μM) caused tension with increasing F_{340}/F_{380} in a concentration-dependent manner (Fig. 1a). 8-iso-PGF_{2 α} -induced contraction was observed initially at 0.3 μM , and the maximal effect of this agent was observed at 10 μM , whereas, F_{340}/F_{380} induced by 8-iso-PGF_{2 α} was augmented initially at 0.1 μM , and the maximal effect of this agent was observed at 1.0 μM (Fig. 1b). In the concentration-response curves for 8-iso-PGF_{2 α} on tension and F_{340}/F_{380} , the values of percent contraction and F_{340}/F_{380} for 10 μM of this agent were $63.8 \pm 10.0\%$ and $84.6 \pm 10.2\%$ of response to 1 μM MCh, respectively ($n = 6$). The values of EC₅₀ for the concentration-response curves for 8-iso-

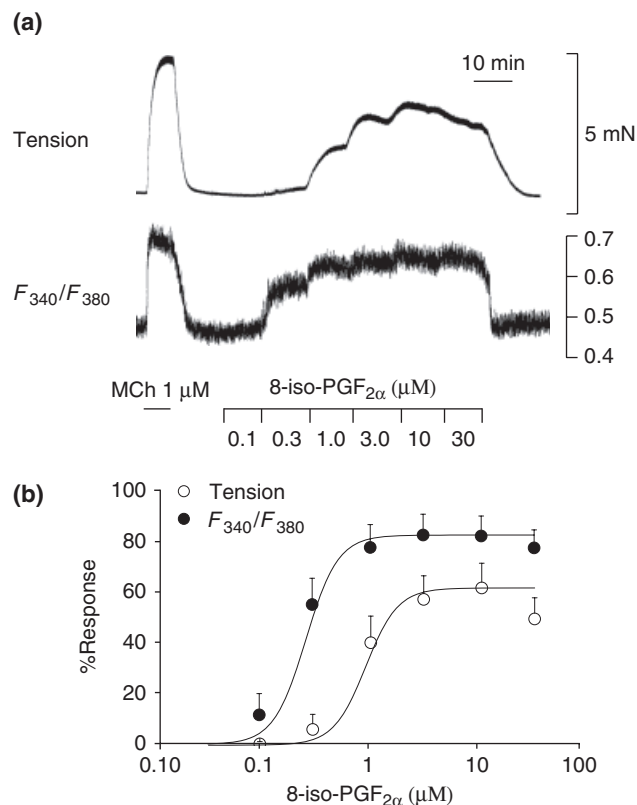


Fig. 1. (a) Representative traces showing effects of cumulative application of 8-iso-PGF_{2 α} (0.1–30 μM) on tension (upper trace) and F_{340}/F_{380} (lower trace). (b) Concentration-response curves for 8-iso-PGF_{2 α} in tension (open circles) and F_{340}/F_{380} (closed circles) induced by 8-iso-PGF_{2 α} . Values of percent contraction and F_{340}/F_{380} were determined by taking the resting condition as 0%, and those by 1 μM MCh in the control condition were taken as 100%. Abscissa expresses molar concentrations in a log scale. MCh, methacholine.

PGF_{2 α} in tension and F_{340}/F_{380} were 1.4 ± 0.6 and 0.3 ± 0.1 μM , respectively ($n = 6$).

Inhibitory effects of a TP receptor antagonist on isoprostane-induced contraction

When ICI-192605 (0.3–30 μM) was cumulatively applied to the fura-2 loaded tissues pre-contracted by 3 μM 8-iso-PGF_{2 α} , ICI-192605 caused an inhibition in tension with reducing F_{340}/F_{380} in a concentration-dependent fashion (Figs 2a and b). The values of IC₅₀ for the curves in tension and F_{340}/F_{380} were 2.2 ± 1.0 and 1.6 ± 0.9 nM, respectively ($n = 10$). ICI-192605 also caused a concentration-dependent inhibition in both tension and F_{340}/F_{380} induced by 0.1 μM U46619, similar to those caused by 8-iso-PGF_{2 α} (Figs 2a and b). The values of IC₅₀ for the curves in tension and F_{340}/F_{380} were 2.5 ± 1.1 and 2.0 ± 0.8 nM, respectively ($n = 10$, not significant). However, ICI-192605 caused modest inhibitions against tension and F_{340}/F_{380} by 1 μM PGF_{2 α} in contrast to those from 8-iso-PGF_{2 α} and U46619 ($n = 6$, $P < 0.05$, Figs 2a and b).

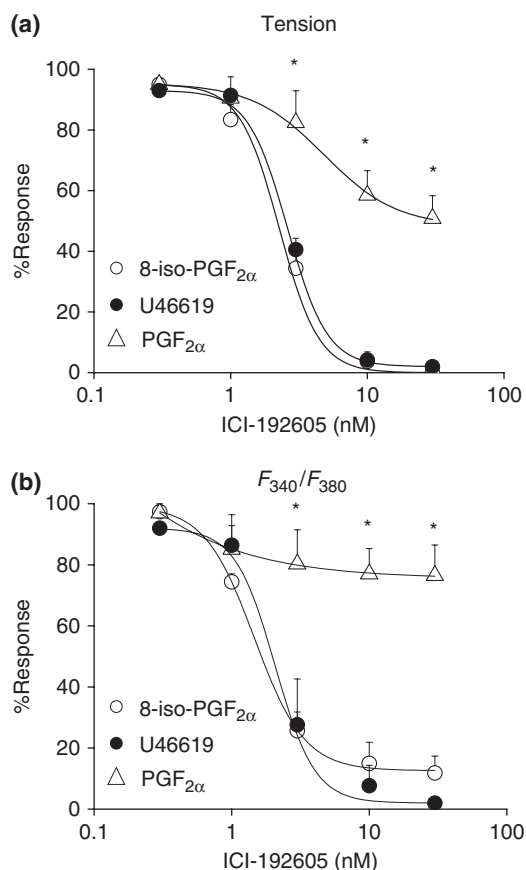


Fig. 2. Effects of TP receptor inactivation against tension and $[Ca^{2+}]_i$ induced by 8-iso-PGF_{2α}. Concentration-inhibition curves for ICI-192605, an inhibitor of TP receptors, in tension (a) and F_{340}/F_{380} (b) induced by 3 μ M 8-iso-PGF_{2α} (open circles), 0.1 μ M U46619 (closed circles), and 1 μ M PGF_{2α} (open triangles). Values of percent contraction and F_{340}/F_{380} in the resting state were taken as 0%, and those values by 3 μ M 8-iso-PGF_{2α} were taken as 100%. Abscissa expresses molar concentrations in a log scale. * $P < 0.05$.

Mechanisms of Ca^{2+} mobilization by 8-iso-PGF_{2α}

Application of 100 μ M SKF96365 resulted in marked attenuations of tension and F_{340}/F_{380} induced by 3 μ M 8-iso-PGF_{2α} (Fig. 3a). The values of percent contraction and F_{340}/F_{380} for 3 μ M 8-iso-PGF_{2α} with 100 μ M SKF96365 inhibition were $22.4 \pm 5.8\%$ and $18.7 \pm 8.4\%$, respectively ($n = 6$). In contrast, application of 10 μ M diltiazem resulted in a reduction of F_{340}/F_{380} by 3 μ M 8-iso-PGF_{2α} with a modest inhibition of contraction (Fig. 3b). The values of percent contraction and F_{340}/F_{380} for 3 μ M 8-iso-PGF_{2α} with 10 μ M diltiazem inhibition were $81.9 \pm 6.8\%$ and $37.4 \pm 9.6\%$, respectively ($n = 6$). When SKF96365 (1.0–100 μ M) was cumulatively applied to the tissues pre-contracted by 3 μ M 8-iso-PGF_{2α}, it markedly inhibited tension with reducing F_{340}/F_{380} in a concentration-dependent manner (Figs 3c and d). In the concentration-inhibition curves for tension and F_{340}/F_{380} , the values of IC_{50} for SKF96365 were 35.1 ± 10.9 and 28.6 ± 9.8 μ M, respectively ($n = 6$). When

diltiazem and verapamil were cumulatively applied in the same way, diltiazem caused a concentration-dependent inhibition in contraction and F_{340}/F_{380} , similar to that induced by verapamil (Figs 3c and d). However, reduced contraction by both diltiazem and verapamil was modest compared with that by SKF96365, with their minimal values of percent contraction being $80.3 \pm 7.2\%$ ($n = 8$) and $78.4 \pm 7.5\%$ ($n = 8$), respectively ($P < 0.05$, Fig. 3c). On the other hand, in the concentration-inhibition curves for F_{340}/F_{380} , the values of IC_{50} for diltiazem and verapamil were 2.1 ± 1.1 ($n = 8$) and 2.5 ± 1.1 μ M ($n = 8$), respectively (Fig. 3d). These values for these VDC inhibitors were much less than that value for SKF96365 ($P < 0.05$, Fig. 3d). These VDC channel inhibitors were more potent than SKF96365 in reducing F_{340}/F_{380} induced by 8-iso-PGF_{2α}. However, SKF96365 was more potent in inhibiting tension than verapamil and diltiazem. When the concentration of extracellular Ca^{2+} was decreased from 2.4 to 0 mM (nominally free), tension and F_{340}/F_{380} induced by 8-iso-PGF_{2α} were completely suppressed (Fig. 4). However, reduced responsiveness to 8-iso-PGF_{2α} was reversed to the control level by adding 2.4 mM Ca^{2+} to the extracellular side (Fig. 4).

Involvement of PC-PLC in the effect of 8-iso-PGF_{2α}

One hundred micromolar of D609 completely inhibited 8-iso-PGF_{2α}-induced contraction with reducing F_{340}/F_{380} (Fig. 5a). The values of percent contraction and F_{340}/F_{380} for 3 μ M 8-iso-PGF_{2α} with 100 μ M D609 were $0.0 \pm 0.0\%$ (complete inhibition) and $23.8 \pm 6.2\%$, respectively ($n = 10$, Fig. 5a). In contrast, 10 μ M U73122 caused modest inhibitions of both contraction and F_{340}/F_{380} induced by 3 μ M 8-iso-PGF_{2α} (Fig. 5b); those values with U73122 were $81.8 \pm 8.6\%$ and $84.1 \pm 10.2\%$, respectively ($n = 10$, Fig. 5b). When D609 (0.1–100 μ M) was cumulatively applied to the tissues pre-contracted by 8-iso-PGF_{2α}, D609 markedly inhibited contraction with reducing F_{340}/F_{380} in a concentration-dependent manner (Figs 5c and d). The values of IC_{50} for D609 in tension and F_{340}/F_{380} were 2.6 ± 1.3 and 14.6 ± 6.5 μ M, respectively ($n = 10$). In contrast, when U73122 (0.1–30 μ M) was applied to the tissues in the same way, U73122 caused a modest inhibition in both tension and F_{340}/F_{380} (Figs 5c and d). The minimal values of percent contraction and F_{340}/F_{380} were $79.1 \pm 7.8\%$ and $80.8 \pm 9.8\%$, respectively ($n = 6$, $P < 0.01$). Moreover, D609 was cumulatively applied in the same way to the tissues pre-contracted by 60 mM K^+ , MCh, and U46619. D609 caused an inhibition in both tension and F_{340}/F_{380} induced by 0.1 μ M U46619 in a concentration-dependent manner, similar to 8-iso-PGF_{2α} (Figs 5e and f). The values of IC_{50} for D609 in tension and F_{340}/F_{380} were 15.2 ± 8.0 and 20.1 ± 8.2 μ M, respectively ($n = 6$). However, D609 did not reduce contraction and F_{340}/F_{380} induced by 60 mM K^+ and 1 μ M MCh (Figs 5e and f). Minimal values of the percent contraction for D609 for the curves were $96.1 \pm 3.5\%$ and $93.8 \pm 5.6\%$, respectively ($n = 6$).

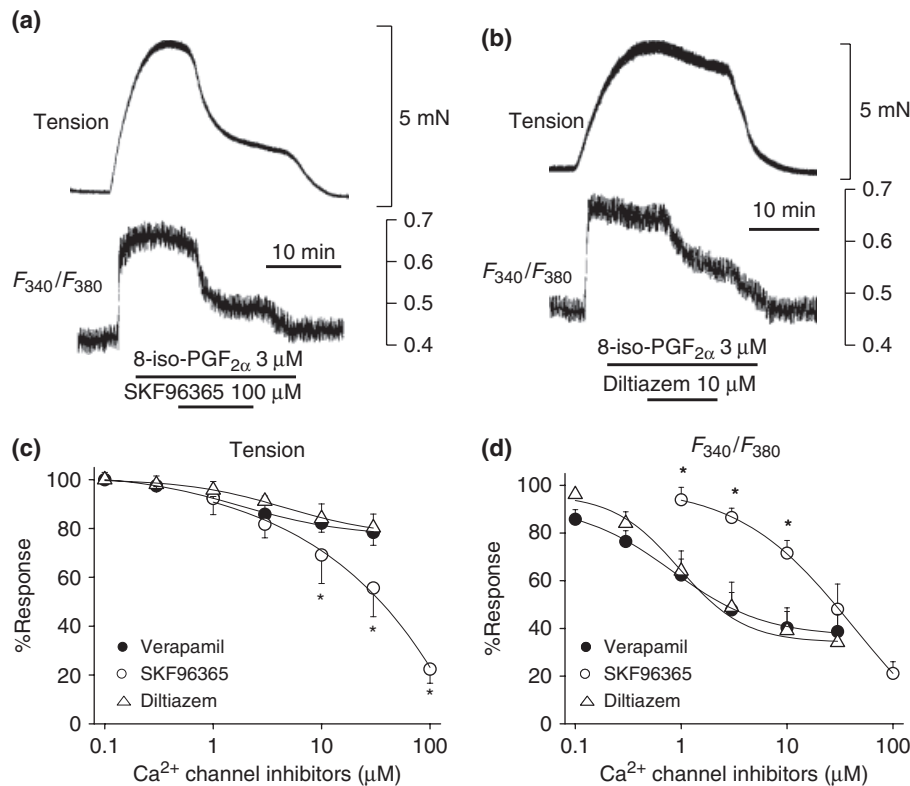


Fig. 3. Representative traces showing inhibitory effects of 100 μM SKF96365 (a) and 10 μM diltiazem (b) in tension (upper trace) and F_{340}/F_{380} (lower trace) induced by 3 μM 8-iso-PGF_{2 α} . Concentration-inhibition curves for SKF96365 (open circles), verapamil (closed circles), and diltiazem (open triangles) against contraction (c) and F_{340}/F_{380} (d) induced by 3 μM 8-iso-PGF_{2 α} . Abscissa expresses molar concentrations in a log scale. Values of percent contraction and F_{340}/F_{380} were determined as shown in Fig. 2. * $P < 0.05$.

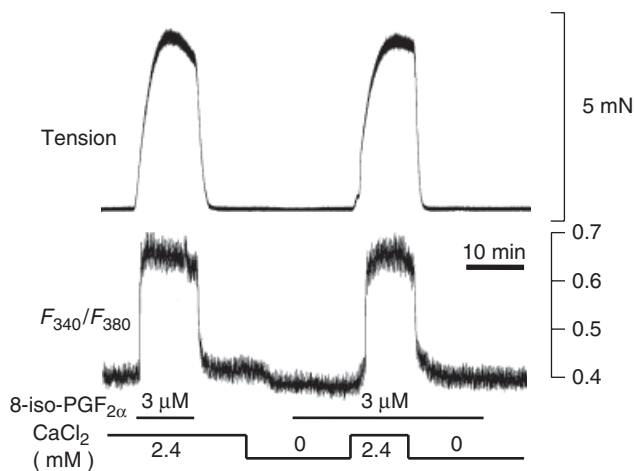


Fig. 4. Representative traces showing effects of removal of extracellular Ca^{2+} on tension (upper trace) and F_{340}/F_{380} (lower trace) induced by 3 μM 8-iso-PGF_{2 α} . 0 mM Ca^{2+} : the nominally free Ca^{2+} solution with 1 mM EGTA, 2.4 mM Ca^{2+} : the normal bathing solution.

Mechanisms of Ca^{2+} sensitization by 8-iso-PGF_{2 α}

Y-27632 markedly reduced tension induced by 3 μM 8-iso-PGF_{2 α} with a modest reduction in F_{340}/F_{380} (Fig. 6a). The values of percent contraction and F_{340}/F_{380} for 8-iso-

PGF_{2 α} with 10 μM Y-27632 were $5.5 \pm 6.3\%$ and $77.3 \pm 11.8\%$, respectively ($n = 6$). In contrast, GF109203X modestly reduced contraction induced by 3 μM 8-iso-PGF_{2 α} with a modest reduction in F_{340}/F_{380} (Fig. 6b). Those values of percent contraction and F_{340}/F_{380} for 8-iso-PGF_{2 α} with 10 μM GF109203X were $63.4 \pm 7.6\%$ and $88.1 \pm 8.0\%$, respectively ($n = 10$). When Y-27632 was cumulatively applied to the tissues pre-contracted by 8-iso-PGF_{2 α} , Y-27632 caused a concentration-dependent inhibition in contraction without a marked reduction in F_{340}/F_{380} (Figs 6c and d). The value of IC_{50} for Y-27632 in tension was $0.4 \pm 0.2 \mu\text{M}$ ($n = 8$). In contrast, when GF109203X and Go6983 were cumulatively applied in the same way, those agents caused a concentration dependent, but a modest inhibition in tension and F_{340}/F_{380} (each $n = 10$, Figs 6c and d). Y-27632 was more potent in inhibiting tension induced by 8-iso-PGF_{2 α} than GF109203X and Go6983 ($P < 0.01$, Fig. 6c), although there was no significant change among the agents in reducing F_{340}/F_{380} .

Discussion

The present results have demonstrated for the first time that Ca^{2+} mobilization mediated by SKF96365- and

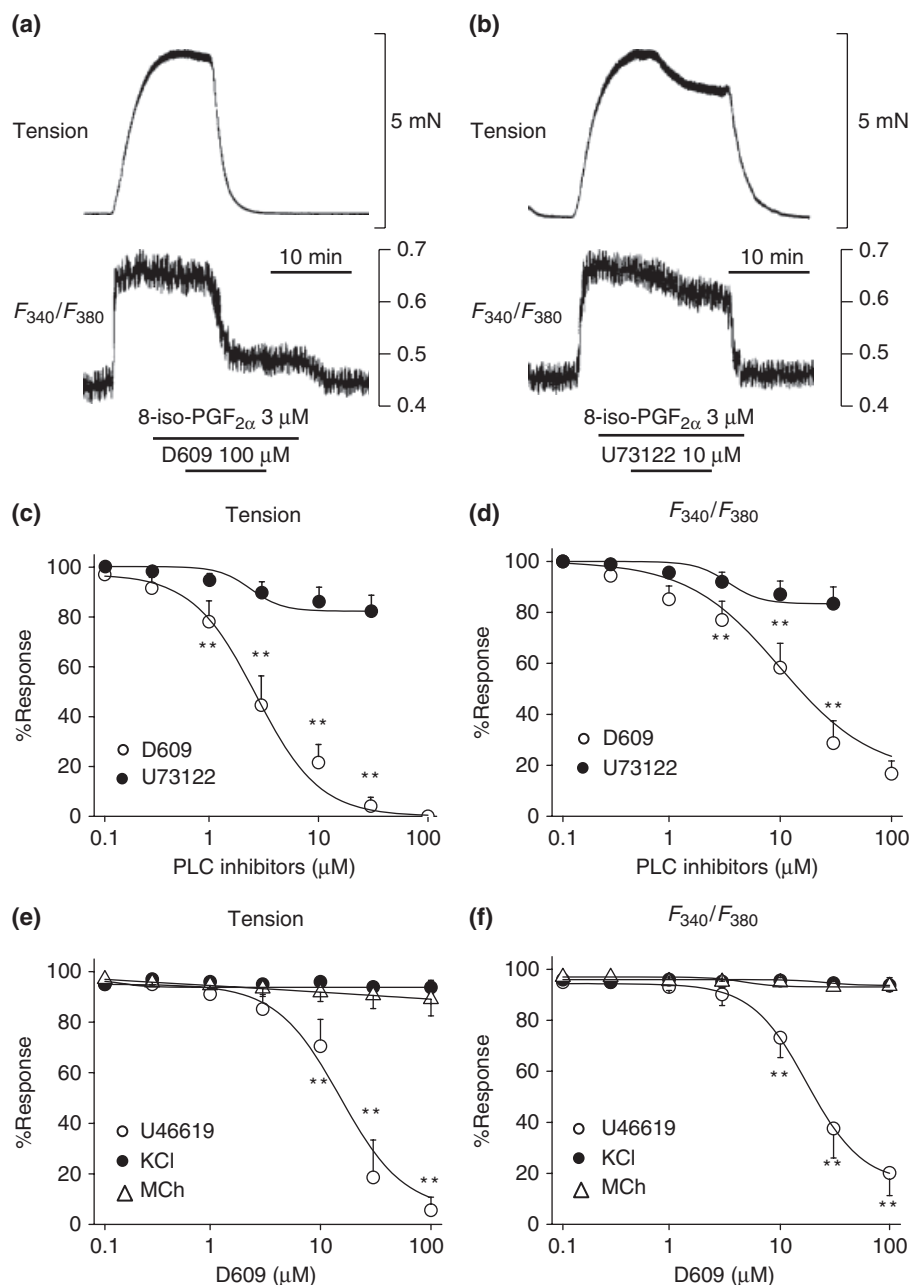


Fig. 5. Representative traces showing effects of 100 μM D609 (a) and 10 μM U73122 (b) on tension (upper trace) and F_{340}/F_{380} (lower trace) induced by 3 μM 8-iso-PGF_{2 α} . Concentration-inhibition curves for D609 (open circles) and U73122 (closed circles) against tension (c) and F_{340}/F_{380} (d) induced by 3 μM 8-iso-PGF_{2 α} . Concentration-inhibition curves for D609 against tension (e) and F_{340}/F_{380} (f) induced by 0.1 μM U46619 (open circles), 1 μM MCh (open triangles), and 60 mM K⁺ (closed circles). Abscissa expresses molar concentrations in a log scale. Values of percent contraction and F_{340}/F_{380} were determined as shown in Fig. 2. ** $P < 0.01$.

D609-sensitive Ca²⁺ influx plays an important role in contraction induced by 8-iso-PGF_{2 α} . Moreover, Ca²⁺ sensitization mediated by Rho-kinase contributes to contraction induced by 8-iso-PGF_{2 α} , and a Rho-kinase inhibitor is effective in suppressing it, independently of [Ca²⁺]_i (Fig. 7).

Tension and [Ca²⁺]_i induced by 8-iso-PGF_{2 α} were markedly attenuated by ICI-192605, an antagonist of TP

receptors, similar to those by U46619, a TXA₂ analog (Figs 1 and 2). Although, as shown in Fig. 2, the action of 8-iso-PGF_{2 α} is mediated by TP receptors [6], the Ca²⁺ signalling pathways involved in this phenomenon have not been fully elucidated. Our observations have demonstrated that Ca²⁺ mobilization plays an important role in the airway smooth muscle contraction induced by 8-iso-PGF_{2 α} (Fig. 1). The response to 8-iso-PGF_{2 α} in the peripheral airway

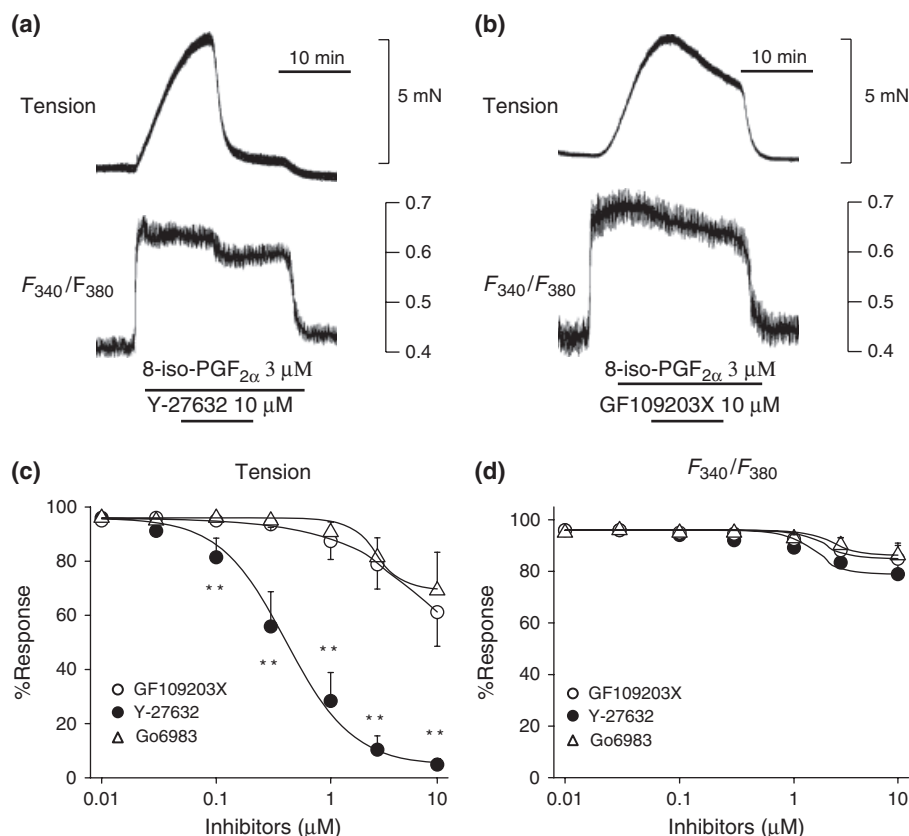


Fig. 6. Representative traces showing inhibitory effects of 10 μM Y-27632 (a) and 10 μM GF109203X (b) on tension (upper trace) and F_{340}/F_{380} (lower trace) induced by 3 μM 8-iso-PGF $_{2\alpha}$. Concentration-inhibition curves for Y-27632 (open circles), GF109203X (closed circles), and Go6983 (open triangles) against tension (c) and F_{340}/F_{380} (d) induced by 3 μM 8-iso-PGF $_{2\alpha}$. Abscissa expresses molar concentrations in a log scale. Values of percent contraction and F_{340}/F_{380} were determined as shown in Fig. 2. ** $P < 0.01$.

smooth muscle has not been examined in this study, because the response to 8-iso-PGF $_{2\alpha}$ has been found to produce no significant change between small and large airways in canines [7].

SKF96365, a non-selective blocker of Ca^{2+} channels, markedly reduced tension and $[\text{Ca}^{2+}]_i$ induced by 8-iso-PGF $_{2\alpha}$ (Fig. 3a). In contrast, although diltiazem and verapamil reduced $[\text{Ca}^{2+}]_i$, the inhibitory effect of these VDC channel inhibitors on tension was not as drastic (Figs 3b–d); those inhibitors maximally reduced the 8-iso-PGF $_{2\alpha}$ -induced contraction by approximately 20%, however, their inhibitory effect was much less than that by SKF96365 (Fig. 3c). These results indicate that Ca^{2+} influx passing through VDC channels is not closely connected with the action of 8-iso-PGF $_{2\alpha}$ in airway smooth muscle contraction. The inhibitory action of those VDC channel inhibitors on U46619-induced contraction was also modest, similar to that on 8-iso-PGF $_{2\alpha}$ (data not shown). Our observations are inconsistent with those of some recent reports shown that U46619-induced contraction is markedly attenuated by VDC channel inhibitors such as nifedipine and nicardipine in vascular smooth muscle [9, 10].

Under the condition of removal of Ca^{2+} at the extracellular side, the first application of 8-iso-PGF $_{2\alpha}$ did not affect either the basal tone or $[\text{Ca}^{2+}]_i$ (Fig. 4), whereas, the first application of carbachol, a muscarinic receptor agonist, to the Ca^{2+} free medium caused a transient contraction [16]; moreover, that contraction was attenuated in the presence of U73122, an inhibitor of PI-PLC (data not shown). These results indicate that Ca^{2+} influx is indispensable for the airway smooth muscle contraction induced by 8-iso-PGF $_{2\alpha}$, while that Ca^{2+} release from sarcoplasmic reticulum (SR) regulated by PI-PLC is not. The involvement of Ca^{2+} mobilization via PLC activity differs between TP and muscarinic receptors activation in airway smooth muscle.

In smooth muscle of various tissues, G-protein coupled receptor (GPCR) agonists have been shown to cause contraction mediated by a sustained Ca^{2+} influx subsequent to Ca^{2+} release from SR via inositol 1,4,5-triphosphate [17]. Because TP receptors also belong to the GPCRs, PI-PLC may be involved in the action on 8-iso-PGF $_{2\alpha}$ as a previous report has demonstrated that U73122 markedly suppresses serotonin-induced contraction of cerebral arteries [18]. However, as shown in Fig. 5,

been fully developed. Although the clinical relevance of the present observations remains unknown, Rho-kinase may prove to be likely a therapeutic target for the physiologic abnormalities induced by oxidative stress in the airway.

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