Pressor responses to platelet-activating factor and thromboxane are mediated by Rho-kinase

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Platelet-activating factor (PAF) contracts smooth muscle of airways and vessels primarily via release of thromboxane. Contraction of smooth muscle is thought to be mediated either by calcium and inositol trisphosphate (IP3)-dependent activation of the myosin light chain kinase or, alternatively, via the recently discovered Rho-kinase pathway. Here we investigated the contribution of these two pathways to PAF- and thromboxane receptor-mediated broncho- and vasoconstriction in two different rat models: the isolated perfused lung (IPL) and precision-cut lung slices. Inhibition of the IP3 receptor (1–10 μM xestospongin C) or inhibition of phosphorylatedinositol-specific PLC (30 μM L-108) did not affect bronchoconstriction but attenuated the sustained vasoconstriction by PAF. Inhibition of myosin light chain kinase (35 μM ML-7) or of calmodulin kinase kinase (26 μM STO609), which regulates the phosphorylation of the myosin light chain, had only a small effect on PAF- or thromboxane-induced pressor responses. Similarly, calmidazolium (10 μM), which inhibits calmodulin-dependent proteins, only weakly reduced the airway responses. In contrast, Y-27632 (10 μM), a Rho-kinase inhibitor, attenuated the thromboxane release triggered by PAF and provided partial or complete inhibition against PAF- and thromboxane-induced pressor responses, respectively. Together, our data indicate that PAF- and thus thromboxane receptor-mediated smooth muscle contraction depends largely on the Rho-kinase pathway.

bronchoconstriction; vasoconstriction; perfused lung; precision-cut lung slices

PLATELET-ACTIVATING FACTOR (PAF) is a potent smooth muscle contractant. In the lungs this causes bronchoconstriction and pulmonary hypertension (3). These effects of PAF are largely mediated by thromboxane and to a smaller part by leukotrienes (53). The signaling pathways responsible for these pressor responses in the lungs are only poorly defined. Smooth muscle contraction is mediated by phosphorylation of myosin light chain (MLC20) by myosin light chain kinase (MLCK) in a calcium-dependent fashion. Traditionally, it is assumed that part of the elevation in intracellular calcium is mediated by inositol trisphosphate (IP3). More recently, calcium-sensitizing pathways were discovered. It had been recognized for some time that the increase in intracellular calcium does not always correlate with the degree in MLCK activity and that the degree of phosphorylation was higher than expected. This was referred to as the Ca2+ sensitization mechanism (42). Further studies identified Rho (3, 21, 42) as part of the Ca2+ sensitization (12, 13). Subsequently, the downstream effector of Rho, the Rho-associated kinase (Rho-kinase) was shown to be crucial for the Ca2+ sensitization in smooth muscle contraction (48). It was shown that Rho-kinase regulates the phosphorylation state of MLC, leading to contraction via binding to the regulatory unit MYPT1 and inactivation of myosin light chain phosphatase (MLCP) as well as direct phosphorylation of MLC (1, 34). Recently, the presence of two other kinases, the Zip-like kinase (32) and CPI-17 (22), that also can regulate the MLCP were reported, but their functional significance is unknown.

It has been suggested that activation of the thromboxane receptor (TP-R) mediates contraction via the Rho-kinase pathway (8). In extrapulmonary arteries the TP-R agonist U-46619 activated RhoA, and Rho-kinase inhibition relaxed or attenuated U-46619-induced contraction (33, 40). So far, there is only one study that has investigated the role of Rho-kinase in the pulmonary vasculature in response to thromboxane (16). In that study, canine isolated pulmonary vein rings were partly protected from U-46619-induced contraction by Rho-kinase inhibition (16). Thus far, the role of Rho-kinase in PAF- and thromboxane-dependent airway responses is unknown. In addition, the role of Rho-kinase has not been studied in intact lungs. The present study was designed to examine the mechanism of PAF- and thromboxane-induced vascular and airway responses in the lung.

MATERIALS AND METHODS

Animals. Female Wistar rats (220 ± 20 g) were obtained from Harlan Winkelmann (Borchen, Germany). Pentobarbital sodium (Narcoren) was purchased from Wirtschaftsgenossenschaft Deutscher Tierärzte (Hannover, Germany). Calmidazolium chloride, L-108, and 1-(5-iodonaphthalene-1-sulfonyl)-1H-hexahydro-1,4-diazepine-HCl (ML-7) were obtained from Biomol Feinchemikalien (Hamburg, Germany); EGTA, l-α-phosphatidylcholine β-acetyl-χ-O-(octade-9-cis-eyn) (PAF), and (15S,2S)-2-[3-(2-benzimidazolyl-propyl)methylamino]ethyl-6-fluoro-1,2,3,4-tetrahydro-1-isopropyl-2-naphthylmethoxy-acetate dihydrochloride (mibefradil) from Sigma (Deisenhofen, Germany); xestospongin C from Calbiochem (Bad Soden, Germany), 9,11-dideoxy-11α, 9α-epoximethanoprostaglandin F2α (U-46619) from Cayman (Ann Arbor, MI), trans-4-[1(R)-1-aminoethyl]-N4-pyrindinyl-cyclohexane carboxamide (Y-27632), and 7-oxo-7H-benzimidazo (2,1a) benz (de) isouquinoline-3-carboxylic acid acetate (STO609) from Tocris/Biotrend (Cologne, Germany). The animal experiments were approved by the Ministerium für Umwelt, Naturschutz und Landwirtschaft des Landes Schleswig-Holstein.

Isolated perfused rat lung preparation. The rat lungs were prepared and perfused essentially as described recently (52a). Briefly, lungs were perfused at constant hydrostatic pressure (12 cmH2O) through the pulmonary artery, which resulted in a flow rate of ~30 ml/min. As the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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a perfusion medium we used Krebs-Henseleit buffer (37 °C) that contained 0.1% glucose and 0.3% HEPES. The total amount of recirculating buffer was 100 ml. The lungs were suspended by the trachea and were ventilated by negative pressure ventilation with 80 breaths/min and a tidal volume of ~2 ml. Every 5 min a hyperinflation (~20 cmH2O) was performed. Artificial thorax chamber pressure was measured with a differential pressure transducer (Validyne DP 45-14), and air flow velocity with a pneumotachograph tube (Fleisch type 0000) connected to a differential pressure transducer (Validyne DP 45-15). The perfusate flow (Narcometric RT 500) and the arterial and venous pressure (Statham P23BB) were continuously monitored. The pH of the perfusate before entering the lung was kept at 7.35 by automatic bubbling of the buffer with CO2 as soon as the pH exceeded this value. A weight transducer was integrated into the chamber lid and allowed continuous assessment of lung weight (50). Please note that the data on PAF-induced edema formation, which depends on a mechanism completely different from thromboxane (7, 10, 53), will be reported separately. For determination of pulmonary resistance and compliance, we analyzed the data by fitting the measured data to the law of motion: 

\[ P = \frac{1}{C \cdot V_T} + R_c \cdot \frac{dV}{dt}, \]

where \( P \) is chamber pressure, \( C \) pulmonary compliance, \( V_T \) tidal volume, \( R_c \) airway resistance, and \( t \) time. Vascular resistance (\( R_v \)) was calculated from \( R_v = (P_A - P_V)/Q \), where \( P_A \) and \( P_V \) are arterial and venous pressure, and \( Q \) is the perfuse flow. The values of control lungs for airway resistance and vascular resistance were 0.24 ± 0.03 cmH2O·s/ml and 0.30 ± 0.04 cmH2O·min/ml, respectively.

Experimental design of the perfused lung studies. PAF was always injected as a bolus of 5 nmol (corresponds to 2.5 μl) directly into the perfuse after 30 min of perfusion and comes up to the lungs after 5–6 s. Therefore \( R_{30} \) or \( R_{30} \) represents the control before adding of PAF or U-46619. All other agents were added to the buffer reservoir and reached the lungs within 5 s. Stock solutions of the substances used were made in either perfusate buffer or ethanol. For experiments with reduced calcium, modified medium was applied from the beginning. We perfused the lungs by recirculating the buffer. The resistance was calculated as \( R/R_{30} \), which represents the ratio of current resistance to control before adding drugs.

Precision-cut lung slices. Precision-cut lung slices were prepared from isolated perfused lungs (IPL) as ascribed (25). Briefly, lungs were filled via the trachea with 0.75% low-melting-point agarose solution and put on ice until the agarose had solidified. Lobes were separated, and cores of 0.9 cm in diameter were obtained and cut into ~250-μm-thin slices (Krumdieck tissue slicer; Alabama Research and Development, Munford, AL). Slices were incubated in minimal essential medium (GIBCO Life Technologies, Eggenstein, Germany), and medium was changed frequently during the first 4 h and incubated overnight.

All the slices were immersed in aqueous solution during the experiments, and the interstitial space was thus directly exposed to the medium. In addition, due to the lack of perfusion there is no hydrostatic pressure to drive edema formation. Therefore, in lung slices, interstitial edema cannot occur. Lung slices are particularly useful to study the bronchial and vascular effects of agents such as calmidazolium that in the whole organ cause such severe edema formation that the experiments have to be terminated.

The airways were imaged and digitized with a digital video camera as described before (25). Airway area before addition of U-46619 was defined as 100%. Bronchoconstriction was expressed as the percent decrease in airway area compared with the control airway area. Inhibitors diluted in deionized water or in ethanol were incubated 10 min before addition of U-46619. The final concentration of ethanol was always <0.1% and did not affect the responses to U-46619.

Measurement of thromboxane. Samples taken from the perfusate at the times indicated were stored at −20 °C. Thromboxane \( A_2 \) (TXA2) was assessed as the stable byproduct TXB2 by enzyme immunoassay (Cayman Chemicals, Ann Arbor, MI) according to the manufacturer’s instructions. The cross-reactivity of the detecting antibody was TXB2 100%, 2,3-dinor TXB2 8.2%, prostaglandins <0.5%.

Statistics. Data were analyzed by unpaired Student’s t-test, either one-sided or two-sided as indicated. In case of repeated measurements (time courses), the maximum value of each curve was taken for analysis. The false discovery rate due to multiple comparisons was controlled by the method of Benjamini and Hochberg (14). In case of heteroscedasticity, data were log-transformed before analysis. \( P < 0.05 \) was considered to be significant. Vascular resistance and lung resistance were normalized to the time point of application of the stimulus (PAF, U-46619). For testing differences in vasoconstriction, we used the vascular conductance as parameter, because resistance becomes infinitely large when strong constriction occurs (resulting in calculation errors), whereas the conductance tends to be zero and reflects the physiological situation of no flow.

RESULTS

Perfusion of PAF through the pulmonary artery of isolated rat lungs resulted in a rapid increase in pulmonary and vascular resistance (Fig. 1; for representative recordings, see Ref. 7), indicating bronchoconstriction and vasoconstriction. Pretreatment of isolated lungs with the phosphatidylinositol-specific phospholipase C (PL-PLC) inhibitor L-108 or the IP3 receptor antagonist xestospongin C had no effect on the PAF-induced bronchoconstriction (Fig. 1). Similarly, both inhibitors failed to affect the U-46619-induced airway contraction in rat lung slices, even at a concentration of 10 μM (Fig. 1E). On the vascular side, in isolated lungs, these agents did not affect the initial vasoconstriction, although they reduced the vascular resistance during the second phase. L-108 did not affect the PAF-induced thromboxane release (maximum thromboxane concentration ± SD after 1 min was 766 ± 497 pg/ml, \( n = 5 \), data not shown).

Next we checked the importance of extracellular calcium by perfusing lungs with various calcium concentrations. These experiments were difficult to carry out, because reduced extracellular calcium may enhance vascular permeability (4, 31). Therefore, the lowest possible concentration used was one-tenth of the normally used concentration of 2.5 mM calcium. Reducing the extracellular calcium concentrations attenuated the PAF-induced bronchoconstriction (Fig. 2A) and vasoconstriction (Fig. 2B). Thromboxane release into the perfusate was also partly dependent on extracellular calcium (Fig. 2C).

To elucidate whether the reduction of the pressor responses in lungs perfused with low-calcium-containing buffer was entirely due to the reduced thromboxane release or also due to reduced smooth muscle contractility, we analyzed the effect of the TP-R agonist U-46619 in lung slices, a model where alterations in vascular permeability are of no concern. In calcium-free medium or medium supplemented with 5 mM EGTA, the bronchoconstriction in response to U-46619 was unchanged (Fig. 3). In line with this, mibefradil, an inhibitor of T-type voltage-dependent calcium channels, had no effect on U-46619-induced vaso- and bronchoconstriction, although it partially attenuated responses to PAF (Fig. 4).

Inhibition of MLCK by ML-7 (Fig. 4) had no or only small effects on the PAF- or U-46619-induced pressor responses. To confirm these results we repeated these studies with another MLCK inhibitor (ML-9) and obtained similar results (contraction in lung slices 41.54 ± 8.34). Unfortunately, the role of calmodulin could not be studied in the IPL, because inhibition
of Ca²⁺/calmodulin-induced MLCK activation with calmidazolium chloride resulted in strong edema formation immediately after administration of this inhibitor. Because such edema cannot occur in the lung slices (see MATERIALS AND METHODS), we used this model to investigate the mechanisms of U-46619-induced bronchoconstriction (26). In lung slices, neither MLCK inhibitors nor calmidazolium affected the U-46619-induced bronchoconstriction (Fig. 4), and neither did xestospongin or L-108 (Fig. 1).

MLC₂₀ may not only be activated by the MLCK, but also by calmodulin kinase I. An enzyme called calmodulin kinase kinase activates calmodulin kinase I, which then controls the activity of MLC₂₀ (45). STO609, an inhibitor of the calmodulin kinase kinase, attenuated the PAF- and U-46619-induced pressor response in the IPL, as well as the U-46619-induced bronchoconstriction in lung slices (Fig. 4).

Finally, we examined the role of the Rho-kinase pathway. Pretreatment with Y-27632 largely attenuated both the PAF-induced broncho- and vasoconstriction (Fig. 5, A and B). In addition, it attenuated thromboxane formation (Fig. 5F). Because the PAF-induced pressor responses are mainly due to thromboxane release, we also investigated the effect of Y-27632 on U-46619-induced pressor responses. Pretreatment with the Rho-kinase inhibitor Y-27632 almost completely abolished the U-46619-induced contractions (Fig. 5, C and D). Similar findings were obtained in experiments with precision-cut lung slices (Fig. 5E). In this model, the effects of Y-27632 and xestospongin were not additive. Inhibition of protein

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**Fig. 1.** Effect of L-108 and xestospongin C on platelet-activating factor (PAF)- and U-46619-induced bronchoconstriction (left column) and vasoconstriction (right column). A–D: in isolated perfused rat lungs, PAF was given as a bolus injection of 5 nmol (final concentration 50 nM) 30 min after beginning of the perfusion. L-108 (16 μg/ml, C and D) and xestospongin (Xesto; 1 μM, A and B) were given 10 min before injection of PAF. Airway (R₂; A and C) and vascular resistance (Rᵥ; B and D) was normalized to the time of PAF injection (Rₓ/Rₓ₃₀). PAF: ●, n = 42; L-108 + PAF: ●, n = 3; xestospongin + PAF: ■, n = 3; control (untreated): ○, n = 9. Whereas the maximum in vasoconstriction was not reduced by L-108 or xestospongin pretreatment, the area under the curve for vasoconstriction was significantly reduced by both L-108 and xestospongin pretreatment. [P < 0.05, 1-sided t-test corrected according to Benjamini and Hochberg (14)]. E: airway area in precision-cut lung slices exposed to U-46619 (U46, 10 μM, n = 15), 1 or 10 μM xestospongin/U-46619 (n = 4–5), or 30 μM L-108 (n = 6). All data are means ± SE.
kinase C (PKC) with calphostin C (5 μM) had no effect on the U-46619-induced airway contraction (contraction was 55 ± 19%, n = 4).

DISCUSSION

In the present study we used two different models to investigate the molecular mechanisms of PAF- and thromboxane-dependent pressor responses. In both models the pulmonary microanatomy is maintained and various lung functions can be studied. In the isolated perfused rat lung model, the integrated responses of the whole organ can be studied in great detail under physiological conditions. The advantage of the lung slices model on the other hand is that it allows one to investigate airway responses under cell culture conditions and to examine agents that are too toxic for study in whole intact lungs. Both models clearly showed the pivotal role of the Rho-kinase pathway for thromboxane-mediated pressor responses.

PAF induces broncho- and vasoconstriction primarily by the release of thromboxane, which acts via the TP-R (7, 51). Thus pharmacological analysis of PAF-induced broncho- and vasoconstriction has to take into account the fact that the agents tested may interfere not only with the pressor responses, but also with the production of thromboxane. Unfortunately, the mechanisms by which PAF triggers eicosanoid production are largely unknown. Previously, we have shown that the PAF-induced thromboxane release is largely mediated by cyclooxygenase-1 (52) and is sensitive to quinine (7). In the present study we demonstrated that PAF-induced thromboxane release is attenuated by low extracellular calcium concentrations and also by inhibition of Rho-kinase, but not by inhibition of the PI-PLC or the IP3 receptor. Our findings suggest that PAF-stimulated thromboxane release from lungs depends on extracellular calcium, but not on IP3-mediated calcium release. This is in line with observations in platelets showing that Ca2+ influx in human platelets occurs independently of IP3 (49). The present findings also help us to better understand the inhibitory effect on PAF-induced thromboxane synthesis of quinine (7), which among other things may block IP3 receptors (18), Ca2+ channels (27), and PLA2 (19). Cytosolic PLA2 is a calcium-dependent enzyme that is critical for arachidonic acid and thus thromboxane formation (23). The inhibitory effect of the Rho-kinase inhibitor Y-27632 on thromboxane release was unexpected, because so far studies of the Rho-kinase pathway have largely focused on its effects on MLC phosphorylation. The

![Fig. 2. Effect of reduced extracellular Ca2+ concentration on PAF-induced bronchoconstriction (A), vasoconstriction (B), and thromboxane release (C) in perfused rat lungs. PAF was given as a bolus of 5 nmol (final concentration 50 nM) 30 min after start of the perfusion. A and B: 5 mM Ca2+, n = 3; 2.5 mM Ca2+, n = 4; 1.25 mM Ca2+, n = 3; 0.25 mM Ca2+, n = 7. C: 2.5 mM Ca2+ + PAF, n = 3; 0.25 mM Ca2+ + PAF, n = 3. Data are means ± SE. Pressor responses and thromboxane B2 (TXB2) release were significantly reduced by low-Ca2+-containing buffer. Rx (A) and Rx (B) were normalized to the time of PAF injection (Rx/Rx0). [P < 0.05, 1-sided t-test corrected according to Benjamini and Hochberg (14).]

![Fig. 3. Effect of extracellular calcium on U-46619-induced bronchoconstriction in precision-cut lung slices. Lung slices were incubated with U-46619 (10 μM) in regular MEM (left bar, n = 26), medium without calcium (center bar, n = 5), or medium in which extracellular calcium was depleted by addition of 5 mM EGTA (right bar, n = 9). Data are means ± SE.

*Fig. 3. Effect of extracellular calcium on U-46619-induced bronchoconstriction in precision-cut lung slices. Lung slices were incubated with U-46619 (10 μM) in regular MEM (left bar, n = 26), medium without calcium (center bar, n = 5), or medium in which extracellular calcium was depleted by addition of 5 mM EGTA (right bar, n = 9). Data are means ± SE.*
present findings add to the growing list of examples showing that the significance of Rho-kinase extends beyond control of smooth muscle contraction (36, 39, 41, 43).

In leukocytes and mesangial cells, thromboxane activates the PI-PLC, which has been linked to increased IP$_3$ concentrations and subsequent release of intracellular calcium from intracellular stores (20, 35, 54). However, neither in perfused lungs nor in lung slices did we find evidence for the involvement of the classic PI response, as both L-108 and xestospongin were ineffective. Notably, the concentration of xestospongin used was at least threefold above its IC$_{50}$ (9, 30), and at this concentration xestospongin prevented the acetylcholine-induced calcium signaling in lung slices (2) and attenuated PAF-induced edema formation in isolated lungs (11). These findings seem to contrast with the widely held assumption that TP-Rs contract smooth muscle cells by coupling to the IP$_3$ pathway (6, 24). However, the evidence for this concept is surprisingly small. Most of that evidence has been derived from studies with platelets rather than smooth muscle cells. And even in platelets there is clear evidence that the release of intracellular calcium that follows activation of the TP-R is at least partly independent of IP$_3$ (44). It is also known that the amount of IP$_3$ that is generated by activation of the TP-R is less than the IP$_3$ formed after activation of muscarinic, histamine, or serotonin receptors (18, 44). The molecular understanding of these processes is complicated by the existence of various PI-PLC isoenzymes (17, 38), of different IP$_3$ receptors (5, 37, 55, 56), and the lack of knowledge whether inhibitors such as L-108 and xestospongin distinguish between these isoforms.

**Fig. 4.** Effect of ML-7, STO609, calmidazolium, and mibebradil on PAF- and U-46619-induced bronchoconstriction (left column) and vasoconstriction (right column). A and B: in isolated perfused rat lungs, PAF (PAF alone, _n_ = 42) was given as a bolus injection of 5 nmol (final concentration 50 nM) 30 min after beginning of the perfusion. C and D: in isolated perfused rat lungs, U-46619 (U-46619 alone, _n_ = 5) was given as a bolus injection of 5 nmol (final concentration 50 nM) 30 min after beginning of the perfusion. ML-7 (16 _µg/ml, _n_ = 4), STO609 (10 _µg/ml, _n_ = 4), and mibebradil (10 _µM, _n_ = 6) were given 10 min before injection of PAF: control (Cont) was _n_ = 12. The maximum $R_{\text{X}}$ (A and C) and $R_{\text{X}}$ (B and D) were normalized to the time of PAF injection ($R_{\text{X}}$/$R_{\text{X0}}$). E: contraction of airway area in precision-cut lung slices exposed to U-46619 (10 _µM, _n_ = 15) pretreated with ML-7 (35 _µM, _n_ = 12), STO609 (26 _µM, _n_ = 6), mibebradil (10 _µM, _n_ = 3), or calmidazolium (10 _µM, _n_ = 6). Data are means ± SE. * _P_ < 0.05 compared with PAF or U-46619, 1-sided _t_-test corrected according to Benjamini and Hochberg (14). All data are means ± SE. The resistance was calculated as $R_{\text{X}}$/$R_{\text{X0}}$, which represents the ratio of current resistance to control before adding drugs.

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The thromboxane-mediated bronchoconstriction appears to occur not only independently of IP₃, but also independently of extracellular calcium (Fig. 3). These studies were performed in precision-cut lung slices, because in this model extracellular calcium can be removed without edema formation (Fig. 3). Our finding that U-46619-induced airway contraction is independent of extracellular calcium is in line with reports showing that U-46619-induced contraction of rat aorta is at least partly mediated by a mechanism independent of extracellular calcium (47). Also in line with these observations is the finding that the T-type calcium channel blocker mibefradil had no effect on U-46619-induced pressor responses, although it slightly affected the responses to PAF, thus suggesting that T-type calcium channels might facilitate thromboxane formation.

Smooth muscle contraction depends on phosphorylation of the MLC₂₀, which may be mediated by MLCK or by calmodulin kinase I as part of the so-called calmodulin kinase pathway (45). Both enzymes depend on intracellular calcium and the calcium-binding protein calmodulin. However, neither inhibition of calmodulin by calmidazolium nor inhibition of MLCK with ML-7 or ML-9 had any effect on U-46619-induced bronchoconstriction, although ML-7 slightly attenuated U44619-induced vasosconstriction and the bronchoconstriction in response to PAF. On the other hand, a consistent attenuation of both PAF- and U-46619-induced pressor responses was observed by pretreatment with the calmodulin kinase kinase inhibitor STO609. At present, this moderate inhibitory effect of STO609 is difficult to explain, since the experiments with calmidazolium indicate that calmodulin plays no role in U-46619-induced bronchoconstriction. Further studies will be required to understand this apparent contradiction.

None of the pathways and signaling molecules discussed above could adequately explain the PAF- and thromboxane-induced pressor responses. The exclusion of these pathways corroborates the major finding of this study, namely that these pressor responses are largely dependent on the Rho-kinase pathway. Our evidence is based largely on the effects of Y-27632, a substance originally developed as a smooth muscle relaxant. It was reported to completely inhibit agonist-induced

![Graphs and images](https://example.com/graphs.png)
contraction of vascular and smooth muscle by selectively inhibiting the Ca$^{2+}$-sensitizing mechanism (48). In the present study, pretreatment with Y-27632 largely attenuated the effects of PAF in airways and vessels and completely abrogated the responses to U-46619. A central role of the Rho-kinase pathway in TP-R-triggered smooth muscle contraction is also supported by a recent study on the spasmodenic effects of isoprostanones in human and porcine bronchial artery smooth muscle (46). So far, few unspacific side effects of Y-27632 have become known, although it was reported to inhibit PKC 

In summary, we have shown that PAF-mediated pressor responses depend on extracellular calcium and the Rho-kinase pathway. Rho-kinase appears to play a dual role in that it effects of Y-27632. However, because calphostin C, an inhibitor of PKC, had no effect on U-46619-induced bronchoconstriction in the lung slices, we believe that inhibition of PKC cannot explain the effects of Y-27632.

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