The effects of the Rho-kinase inhibitor Y-27632 on arachidonic acid-, GTPγS-, and phorbol ester-induced Ca^{2+}-sensitization of smooth muscle

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Abstract The effects of the Rho-kinase inhibitor, Y-27632 [1] on Ca^{2+}-sensitization of force induced by arachidonic acid (AA), phorbol 12,13-dibutyrate (PDBu), GTPγS, and by the stable thromboxane analog, 9,11-dideoxy-9α,11α-methanoepoxy-$PGF_{2\alpha}$ (U-46619), were determined in α-toxin-permeabilized smooth muscles. Y-27632 relaxed (up to 99%) Ca^{2+}-sensitization by GTPγS (10 μM) and U-46619 (1 μM), but not by PDBu (20 μM), and reduced GTPγS-induced myosin light chain (MLC_{20}) phosphorylation from 28% to 17% ($P = 0.002$). GTPγS-induced force sensitization was inhibited by Y-27632 more potently when the inhibitor was added during the plateau of force than prior to stimulation. In α-toxin-permeabilized smooth muscle, Y-27632 inhibited AA (50 μM)-induced Ca^{2+}-sensitization of force (by 66 ± 1.3%) and reduced MLC_{20} phosphorylation. In contrast, Y-27632 did not relax force Ca^{2+}-sensitized by AA in smooth muscle permeabilized with Triton X-100. We conclude that (i) AA induces Ca^{2+}-sensitization through dual mechanisms, one mediated by Rho-kinase (or a related kinase), and (ii) Rho-kinase is not required for phorbol ester-induced Ca^{2+}-sensitization.

Key words: Signal transduction; Rho; Kinase C; Phosphatase

1. Introduction

The level of regulatory myosin light chain (MLC_{20}) phosphorylation that is the primary determinant of smooth muscle contraction can be modulated independently of $[Ca^{2+}]_i$ (review in [2]) by up- or downregulating the activities of myosin light chain kinase (MLCK) and smooth muscle myosin phosphatase (SMPP-1M). Inhibition of MLCK and activation of SMPP-1M, at a given $[Ca^{2+}]_i$, decrease the level of MLC_{20} phosphorylation and thereby desensitize the contractile apparatus to Ca^{2+} [3,4], whereas inhibition of SMPP-1M sensitizes it [2,5–7]. Upstream initiators of Ca^{2+}-sensitization include agonist-activated excitatory serpentine receptors coupled to heterotrimeric G-proteins, agents (guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS), AlF_4^-) that directly activate G-proteins (review in [7]), activators (bryostatin and phorbol esters) of conventional and novel protein kinase Cs (PKCs) [8,9], and arachidonic acid (AA) [10–12]. Inhibition of SMPP-1M, achieved through a variety of parallel pathways, is the major downstream Ca^{2+}-sensitization mechanism ([5,8,13–16]; review in [2,7]). Inhibition of SMPP-1M may be direct, through phosphorylation of its regulatory subunit ([17,18]; review in [6]), or indirect, by kinase C-mediated phosphorylation and consequent activation of a protein phosphatase 1C (PP1-C) phosphatase inhibitor [5,19,20].

The monomeric GTPase, RhoA, plays a major role in Ca^{2+}-sensitization, and its inactivation by bacterial exoenzymes inhibits or blocks Ca^{2+}-sensitization ([21], and refs. therein; [22–24]). Active RhoA (RhoA·GTP) activates Rho-kinases, serine-threonine kinases that can phosphorylate the regulatory subunit of SMPP-1M and inhibit its catalytic activity [18], but whether Rho-kinases are the sole G-protein-coupled effectors of Ca^{2+}-sensitization and the relationship between reversal of Rho-kinase-mediated Ca^{2+}-sensitization of force and dephosphorylation of MLC_{20} have not been fully determined. We used a recently developed [1] highly selective inhibitor of Rho-kinase, Y-27632, to determine whether Rho-kinase was an effector of Ca^{2+}-sensitization induced by AA and phorbol esters and whether relaxation induced by Y-27632 was associated with dephosphorylation of MLC_{20}.

2. Materials and methods

Force was measured on small strips of rabbit (2–3 kg) main or branches of pulmonary artery with the endothelium removed by gentle scraping (1–2 mm long, 300–400 μm wide) or portal vein (3 mm long, 200–300 μm wide) at 20–22°C as described previously [25].

After recording contractions evoked by high K^+ and by agonists, the strips were incubated in relaxing solution (G1) containing 1 mM EGTA and 4.5 mM MgATP and permeabilized with *Staphylococcus aureus* α-toxin [25–27]. For Triton X-100 permeabilization, 0.05% (pulmonary artery) or 0.1% (portal vein) Triton X-100 was applied to the p*Ca* 6.0 solution for 20 or 10 min, respectively, in the presence of 1 μM calmodulin (CaM) that was included in all solutions used after Triton X-100 permeabilization. Forces were normalized to maximal Ca^{2+} (p*Ca* 4.5)-induced contraction.

For MLC_{20} phosphorylation measurement by two-dimensional isoelectric focusing/SDS-PAGE electrophoresis and densitometry of the colloidal gold-stained MLC_{20} [26], strips of α-toxin-permeabilized main branches of pulmonary artery were stimulated with 5 μM GTPγS at p*Ca* 7 for 12 min. When force had reached a plateau, Y-27632 was applied for 19 min, leading to relaxation to baseline, and then frozen. GTPγS (5 μM) stimulated (without Y-27632) time-matched strips were similarly frozen. Non-muscle myosin that is present in tonic smooth muscles and co-migrates with doubly phosphorylated smooth muscle myosin [26,28,29] was not included in the measurements. The effect of Y-27632 on AA-sensitized MLC_{20} phosphorylation in portal vein smooth muscle was determined by a protocol designed to minimize limitations due to slow diffusion of AA [12]. After permeabilization with α-toxin, portal vein strips were pre-incubated with AA (50 μM) in 1 mM EGTA containing relaxing solution for 60 min and then stimulated with p*Ca* 6.7 and AA.

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Abbreviations: GEF, guanine nucleotide exchange factor; GDI, guanine nucleotide dissociation inhibitor; PDBu, phorbol 12,13-dibutyrate; GTPγS, guanosine 5′-*O*-(3-thiotriphosphate); U-46619, 9,11-dideoxy-9α,11α-methanoepoxy-$PGF_{2\alpha}$; MLCK, myosin light chain kinase; SMPP-1M, smooth muscle myosin phosphatase; MLC_{20}, 20-kDa regulatory myosin light chain; PKC, protein kinase C; AA, arachidonic acid; PP1-C, protein phosphatase 1C; CaM, calmodulin

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Y-27632 (30 μM) was added at the plateau of the contraction, and strips were frozen when the Y-27632-induced relaxation reached steady state (15 min); p*Ca* 6.7 and AA-stimulated, time-matched controls were similarly frozen. The levels of phosphorylation were determined as described above.

Statistical comparisons were made by unpaired Student's *t*-test. Differences were considered to be significant when $P < 0.05$. Results are given as means ± S.E.M.

α-Toxin was from List Biological Laboratories (Campbell, CA, USA) and phorbol 12,13-dibutyrate (PDBu) from Gibco-BRL (Gaithersburg, MD, USA); Triton X-100 and GTPγS from Boehringer Mannheim (Germany); A23187 from Calbiochem (San Diego, CA, USA); 9,11-dideoxy-9α,11α-methanoepoxy-$PGF_{2\alpha}$ (U-46619) from Cayman Chemical (Ann Arbor, MI, USA); and Y-27632 was a gift from Yoshitomi (Osaka, Japan).

3. Results

3.1. Effect of Y-27632 on U-46619-induced Ca^{2+}-sensitization

In order to evaluate in our preparations the effectiveness of Y-27632 as an inhibitor of Ca^{2+}-sensitization, we first determined its effects on U-46619-induced Ca^{2+}-sensitization of rabbit pulmonary artery, a smooth muscle highly sensitive to G-protein-coupled Ca^{2+}-sensitization by this thromboxane analogue [30].

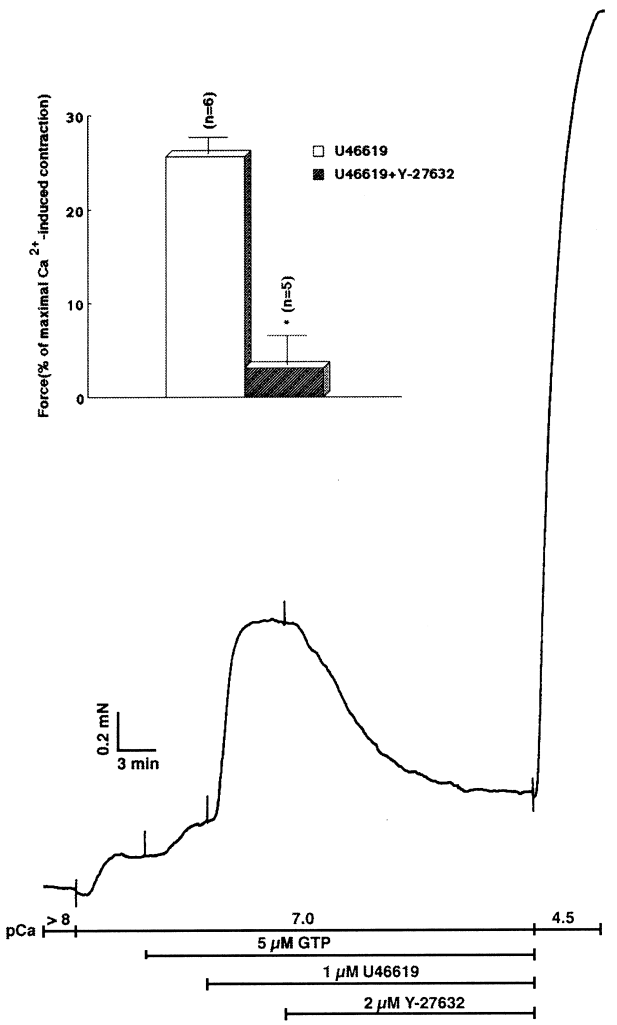

Fig. 1. Abolition of the thromboxane analogue U-46619-induced Ca^{2+}-sensitization by the Rho-kinase inhibitor (Y-27632) in α-toxin-permeabilized main branches of rabbit pulmonary artery at constant Ca^{2+}. Five μM GTP necessary for G-protein-coupled Ca^{2+}-sensitization induced by agonists was present throughout the experiments. Data summary shown in the insert. The sensitization is expressed as the force developed in addition to the response at p*Ca* 7.0. Force is normalized to maximal p*Ca* 4.5-induced contraction (means ± S.E.M.).

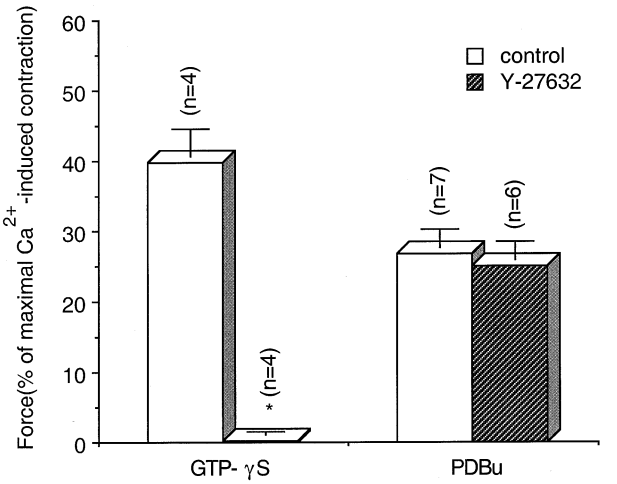

Fig. 2. Summary of GTPγS- or PDBu-induced Ca^{2+}-sensitization in rabbit pulmonary artery permeabilized with α-toxin: effect of the Rho- kinase inhibitor Y-27632. Contractions were induced by 10 μM GTPγS and 20 μM PDBu. Ten μM Y-27632 selectively inhibited the GTPγS-induced contraction (up to 99.2%, $n = 4$), but not the PDBu-induced contraction.

The force developed in response to a maximal concentration (1 μM) of U-46619 in the presence of 5 μM GTP was 26 ± 1.7% and was relaxed by Y-27632 (2 μM) to 3.1 ± 1.3% ($n = 5$; Fig. 1). This result is consistent with those obtained on pig coronary artery and guinea pig tracheal smooth muscles in which Y-27632 relaxed contractions induced by agonists acting on G-protein-coupled receptors: phenylephrine, histamine, acetylcholine, serotonin, endothelin and U-46619 [1].

3.2. The pre- and post-exposure-dependent effects of Y-27632 on GTPγS-induced Ca^{2+}-sensitization of force and MLC_{20} phosphorylation and its lack of effect on PDBu-induced Ca^{2+}-sensitization

Compared to its effectiveness ($K_i = 0.14$ μM) in inhibiting the Rho-kinase p160 ROCK, Y-27632 is a relatively weak inhibitor of conventional protein kinase C (isolated from rat brain); the respective K_i values differ by two orders of magnitude (Table 1, Uehata et al. [1]). To determine whether the conventional and novel PKCs that are activated by phorbol esters are also insensitive to Y-27632 in situ, we compared its effects on, respectively, GTPγS- and PDBu-evoked Ca^{2+}-sensitization of contraction.

Force induced in pulmonary artery by GTPγS (10 μM) at p*Ca* 7.0 was relaxed by Y-27632 (10 μM) from 40 ± 3.9% of maximal force (at p*Ca* 4.5) to 0.3 ± 0.3% (Fig. 2; $n = 4$, $P < 0.001$), as expected [1]. This was associated with reduction of the 10 μM GTPγS-induced increase in MLC_{20} phosphorylation from 28 ± 6.5% ($n = 7$) to 17 ± 4.8% ($n = 6$, $P = 0.002$). In contrast, PDBu (20 μM)-induced force (27 ± 2.7%) was not inhibited (Fig. 2) by Y-27632 (25 ± 2.6%; $n = 6$–7, $P = 0.7$), which was also without effect on Ca^{2+}-sensitization by a lower concentration (5 μM) of PDBu: 26 ± 3.6% and 25 ± 1.4% ($n = 2$–5, $P > 0.9$).

While Ca^{2+}-sensitization of rabbit portal vein by 10 μM

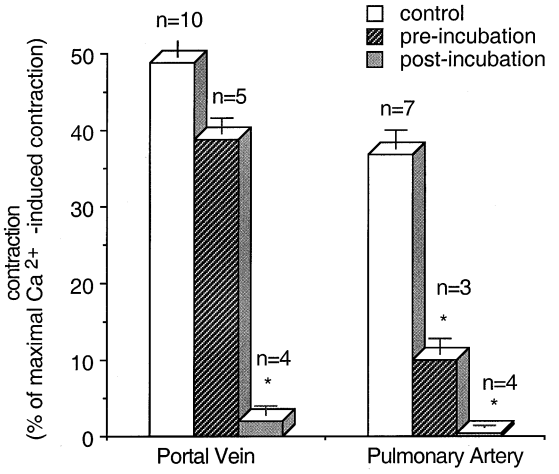

Fig. 3. Y-27632 inhibited GTPγS-induced Ca^{2+}-sensitization of force more effectively when it was added at the steady-state of GTPγS-induced contraction than when it was added prior to the addition of GTPγS. For the pre-incubation with Y-27632, 30 μM (portal vein) or 10 μM (pulmonary artery) Y-27632 was added for 10 min before the addition of GTPγS. Comparisons of GTPγS-induced force values with pre- and post-additions of Y-27632 were highly significantly different, $*P < 0.05$, as well as when the pre- and post-Y-27632 values were compared with each other.

GTPγS was completely relaxed by 30 μM Y-27632 (from 49 ± 5.4% to 2 ± 1.3%, $n = 3$–4; Fig. 3), pre-incubation with 30 μM Y-27632 inhibited the Ca^{2+}-sensitization induced by subsequently adding GTPγS by only 20%, from 49 ± 5.4% ($n = 4$) to 39 ± 2.2% ($n = 5$, $P > 0.05$; Fig. 3). This lowered activity of the inhibitor was not due to its binding or catabolism, because transfer into fresh solution having an equal concentration of Y-27632 (30 μM) did not result in greater relaxation.

3.3. The different, permeabilization-dependent effects of Y-27632 on AA-induced Ca^{2+}-sensitization

Y-27632 concentration-dependently inhibited AA (50 μM)-induced Ca^{2+}-sensitization of force in α-toxin- (Fig. 4), but not in Triton X-100-permeabilized rabbit portal vein, consistent with the participation of the cell membrane in Rho-signaling [21,22]. Furthermore, the relaxant potencies of Y-27632 (tested over 1–30 μM) on AA-induced and GTPγS-induced contraction were similar, although the 30 μM Y-27632-induced relaxation of GTPγS-induced contraction (by 97 ± 2.5%) was more complete than of AA-induced contraction (by 66 ± 1.3%). MLC_{20} phosphorylation was reduced by Y-27632 from 49 ± 3.7% ($n = 11$) to 38 ± 3.4% ($n = 12$, $P < 0.05$).

Vigorous permeabilization of smooth muscle with Triton X-100 or saponin abolishes the Ca^{2+}-sensitizing effect of GTPγS, while retaining some Ca^{2+}-sensitization by AA and by the protein phosphatase inhibitor, microcystin [21]. In contrast to its effect on α-toxin-permeabilized preparations, in Triton X-100-permeabilized smooth muscle Ca^{2+}-sensitization of force by AA was not significantly inhibited by 30 μM Y-27632 (57 ± 4.5% vs. 48 ± 2.3%, $n = 3$ each, $P > 0.05$).

3.4. Different effects of Y-27632 on Ca^{2+}-induced contractions in, respectively, α-toxin- and Triton X-100-permeabilized rabbit pulmonary artery

Inactivation of RhoA with the ADP-ribosylating bacterial exoenzyme, C3, can reduce the amplitude of submaximal Ca^{2+}-induced contractions of smooth muscle permeabilized with β-escin [21,31], possibly as the result of 'constitutive' submaximal activity of the RhoA pathway. Therefore, we determined the effects of Y-27632 on Ca^{2+}-induced tension in α-toxin-permeabilized rabbit pulmonary artery and found it to be $[Ca^{2+}]$-dependent: the lower the concentration of Ca^2, the more extensive the relaxation by Y-27632. In pulmonary artery at p*Ca* 6.0, 10 μM Y-27632 had no effect on Ca^{2+}-induced contractions: forces were 65 ± 3.3% and 57 ± 5.3%, respectively, in the presence or absence of 10 μM Y-27632 ($n = 4$, $P > 0.25$). However, the same concentration of Y-27632 decreased contractions induced by p*Ca* 6.3 from 23 ± 2% ($n = 4$) to 13 ± 1.9% ($n = 4$, $P < 0.005$). Y-27632 30 μM also inhibited submaximal Ca^{2+}-induced contraction of rabbit portal vein permeabilized with α-toxin from 19 ± 2.6% ($n = 5$) to 1.4 ± 0.7% ($n = 5$, $P < 0.05$).

To determine whether the above effects of Y-27632 were

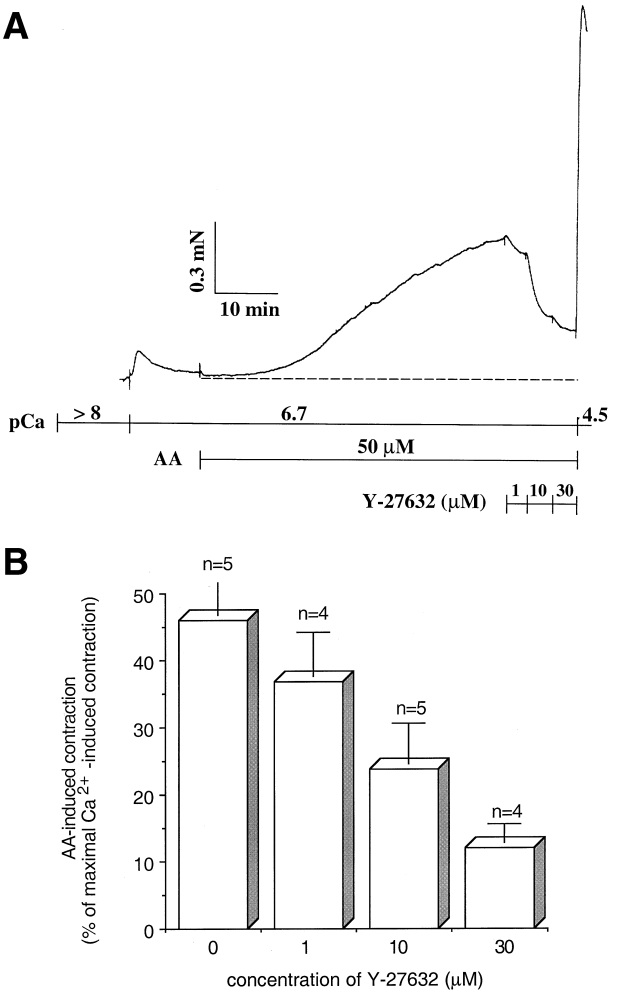

Fig. 4. A: Incomplete inhibition of AA-induced Ca^{2+}-sensitization of force by the Rho-kinase inhibitor Y-27632. α-Toxin-permeabilized rabbit portal vein strips were first exposed to submaximal Ca^{2+}, which gave rise to a small contraction of only 4.3% of the maximal p*Ca* 4.5 contraction. Fifty μM AA was then added. B: Summary of concentration-dependent relaxation of AA-induced Ca^{2+}-sensitization of force by Y-27632 in α-toxin-permeabilized rabbit portal vein. Various concentrations of Y-27632 were added cumulatively after the AA-induced contraction reached steady-state and the magnitude of relaxation measured.

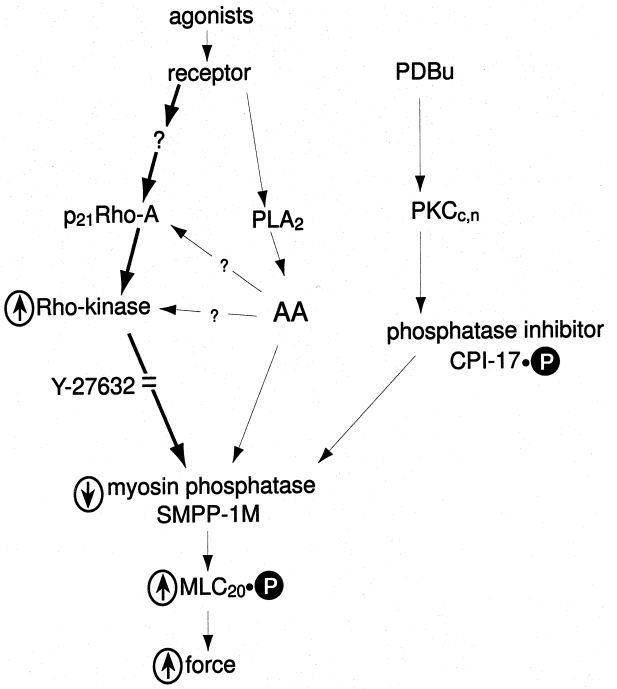

Fig. 5. Ca^{2+}-sensitizing pathways in smooth muscle. Force developed by smooth muscle is proportional, albeit not necessarily linearly, to the level of MLC_{20} phosphorylation by Ca^{2+}-calmodulin-dependent MLCK. A major physiological Ca^{2+}-sensitizing mechanism is initiated by the binding of an excitatory agonist to its receptor, activation and translocation of $p21^{rhoA}$ to the cell membrane [22], and activation of Rho-kinase by Rho. Phosphorylation by Rho-kinase inhibits SMPP-1M, resulting in increased MLC_{20} phosphorylation in the continued presence of MLCK activity. This pathway can also be activated by AA, as indicated by the partial inhibition of the Ca^{2+}-sensitizing effect of AA on force by the Rho-kinase inhibitor, Y-27632. AA can also inhibit SMPP-1M through another mechanism that is not inhibited by Y-27632. Phorbol ester-activated conventional and novel kinase Cs ($PKC_{c,n}$) phosphorylate and so activate the SMPP-1M inhibitor, CPI-17 [20], and inhibit dephosphorylation of MLC_{20} independently of Rho-kinase. Thus several different upstream mechanisms converge to inhibit dephosphorylation of MLC_{20}, resulting in Ca^{2+}-sensitization. SMPP-1M, smooth muscle myosin phosphatase; MLCK, myosin light chain kinase; MLC_{20}, regulatory smooth muscle myosin light chain; MLC_{20p}, MLC_{20} phosphorylated on the serine 19; PKC, protein kinase C; $CPI\text{-}17_p$, CPI-17 phosphorylated by PKC [20].

non-specific (on MLCK) or reflected the 'constitutive' activation of RhoA, we determined the effect of Y-27632 on Triton X-100-permeabilized smooth muscles that are insensitive to GTPγS [21]. Contractions of Triton X-100-permeabilized strips of rabbit pulmonary artery induced with a $[Ca^{2+}]$ (p*Ca* 6.0) and [CaM] necessary to produce submaximal forces similar to those developed by α-toxin-permeabilized strips at p*Ca* 6.3 were not relaxed by Y-27632: $25 \pm 3.3\%$ ($n = 4$) vs. $24 \pm 3\%$ ($n = 4$, $P = 0.8$). Even increasing the concentration of Y-27632 to 200 μM caused no inhibition of the Ca^{2+}-induced contraction, consistent with the low inhibitory activity of Y-27632 ($K_i > 250$ μM) [1] for the Ca^{2+}-CaM-activated MLCK.

4. Discussion

The inhibition of the Ca^{2+}-sensitizing effects of the thromboxane analogue U-46619 and GTPγS in rabbit vascular smooth muscle by the Rho-kinase inhibitor Y-27632 (Figs. 1 and 2) and its lack of a relaxant effect on Ca^{2+}-induced contractions (in Triton X-100-permeabilized preparations) are consistent with its being a highly selective inhibitor of Rho-kinase, as compared to MLCK [1]. The main new finding of our study is that Ca^{2+}-sensitization by AA, but not by PDBu, may be at least partially mediated by Rho-kinase.

AA, a natural product of phospholipid hydrolysis by phospholipase A2 and/or D, can, at concentrations comparable to those released by GTPγS in vivo, Ca^{2+}-sensitize smooth muscle [10,11]. AA can also activate the atypical kinase C, PKC-ζ [32,33] and AA-induced Ca^{2+}-sensitization of smooth muscle is inhibited by a pseudo-substrate inhibitor of PKC-ζ, whereas a phospholipase A2 inhibitor, ONO-R5-082, inhibits phenylephrine-induced Ca^{2+}-sensitization and AA release [12]. AA can also Ca^{2+}-sensitize extensively detergent-permeabilized smooth muscles not responsive to GTPγS, and selectively inhibits the myosin phosphatase activity of isolated SMPP-1M in solution, probably by directly dissociating the catalytic from the regulatory subunit [10]. The reversal of AA-induced Ca^{2+}-sensitization by Y-27632 in α-toxin-permeabilized smooth muscle (present study) is consistent with a mechanism initiated by AA that involves P160 ROCK [1]. Because Ca^{2+}-sensitization by AA was only partially inhibited by Y-27632 in α-toxin-permeabilized and not significantly in smooth muscle extensively permeabilized with Triton X-100, we suggest that AA can act by a dual mechanism: (i) activation of Rho-kinase (or related kinase) [12,34] directly, and/or by dissociating the RhoA·guanine nucleotide dissociation inhibitor (GDI) complex [35] and acting as a guanine nucleotide exchange factor (GEF) for p21RhoA, similar to its effect on Rac·GDI, and (ii) direct inhibition of SMPP-1M [10]. AA may also contribute to activation of Rho-kinase by agonists that release endogenous AA [11], but in that case it remains to be explained why AA released by PDBu [11] does not have this effect, as indicated by the resistance of PDBu-induced Ca^{2+}-sensitization to Y-27632 (present study) and to ADP-ribosylation of RhoA [22].

Compared to its high potency in reversing GTPγS-induced Ca^{2+}-sensitization, pre-treatment with Y-27632 was, surprisingly, less effective (Fig. 3). We can only speculate that this may indicate a dual site of action of Y-27632 on P160 ROCK: binding at one site of the inactive enzyme may imply inhibition at the catalytic site of the activated form. Alternatively, in situ exposure of SMPP-1M to Y-27632 may reduce its sensitivity to inhibition by Rho-kinase. It is interesting that Y-27632 dramatically reduced the blood pressure in three types of experimentally hypertensive rats, without having a major effect on normotensives [1]. The underlying assumption, then, would be that Rho-kinase was activated in the hypertensives, but not in normotensive controls. Our findings suggest that Y-27632 would reduce excitatory (e.g. α-adrenergic) agonist-induced increases in blood pressure of normotensive animals, but would be less effective in preventing such responses.

Phorbol ester-induced Ca^{2+}-sensitization was resistant to concentrations of Y-27632 that nearly completely blocked the effects of GTPγS (Fig. 2). Thus, although in solution the selectivity of Y-27632 for P160 ROCK compared to brain kinase C is somewhat limited [1], in situ the conventional and novel PKCs that are activated by phorbol esters are not inhibited by concentrations of Y-27632 that inhibit the G-protein-coupled mechanisms. The resistance of PDBu-

induced effects to Y-27632 is consistent with the conclusion that phorbol esters and the conventional and novel PKCs activated by them operate predominantly through different upstream mechanisms than the physiological, G-protein-coupled ones [8,36,37]. A recent preliminary report [38] of airway smooth muscle also implicates P160 ROCK, rather than conventional and novel PKCs, in G-protein-coupled Ca^{2+}-sensitization. The several Ca^{2+}-sensitizing pathways converging on inhibition of SMPP-1M are shown in the scheme of Fig. 5 (see also [2,39]).

The relaxation of submaximal Ca^{2+}-induced contractions of α-toxin-permeabilized smooth muscles by Y-27632 is of technical interest. Our results are consistent with previous studies that have suggested that inhibition of the RhoA pathway could reduce force developed in response to submaximal elevations of Ca^{2+} in smooth muscles permeabilized with β-escin [10], and in one study GDPβS induced a shift in the p*Ca* tension curve [31]. These effects of Y-27632 are not due to partial inhibition of MLCK ($K_i > 250$ μM) [1], because (submaximal) Ca^{2+}-induced tension is not affected in smooth muscles in which the G-protein-coupled pathway was eliminated through extensive permeabilization with Triton X-100 (present study).

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References

[1] Uehata, M., Ishizaki, T., Satoh, H., Ono, T., Kawahara, T., Morishita, T., Tamakawa, H., Yamagami, K., Inui, J., Maekawa, M. and Narumiya, S. (1997) Nature 389, 990–994.
[2] Somlyo, A.P. and Somlyo, A.V. (1994) Nature 372, 231–236.
[3] Gallagher, P.J., Herring, B.P. and Stull, J.T. (1997) J. Muscle Res. Cell Motil. 18, 1–16.
[4] Wu, X., Haystead, T.A.J., Nakamoto, R.K., Somlyo, A.V. and Somlyo, A.P. (1998) J. Biol. Chem. 273, 11362–11369.
[5] Somlyo, A.P., Kitazawa, T., Himpens, B., Matthijs, G., Horiuti, K., Kobayashi, S., Goldman, Y.E. and Somlyo, A.V. (1989) Adv. Proteins Phosphatases 5, 181–195.
[6] Hartshorne, D.J., Ito, M. and Erdödi, F. (1998) J. Muscle Res. Cell Motil. 19, 325–341.
[7] Somlyo, A.P., Wu, X., Walker, L. and Somlyo, A.V. (1998) Rev. Physiol. Biochem. Pharmacol. 134, 203–236.
[8] Jensen, P.E., Gong, M.C., Somlyo, A.V. and Somlyo, A.P. (1996) Biochem. J. 318, 469–475.
[9] Walsh, M.P., Andrea, J.E., Allen, B.G., Clement-Chomienne, O., Collins, E.M. and Morgan, K.G. (1994) Can. J. Physiol. Pharmacol. 72, 1392–1399.
[10] Gong, M.C., Fuglsang, A., Alessi, D., Kobayashi, S., Cohen, P., Somlyo, A.V. and Somlyo, A.P. (1992) J. Biol. Chem. 267, 21492–21498.
[11] Gong, M.C., Kinter, M.T., Somlyo, A.V. and Somlyo, A.P. (1995) J. Physiol. (Lond.) 486, 113–122.
[12] Gailly, P., Gong, M.C., Somlyo, A.V. and Somlyo, A.P. (1997) J. Physiol. 500, 95–110.
[13] Kitazawa, T., Masuo, M. and Somlyo, A.P. (1991) Proc. Natl. Acad. Sci. USA 88, 9307–9310.
[14] Iizuka, K., Dobashi, K., Yoshii, A., Horie, T., Suzuki, H., Nakazawa, T. and Mori, M. (1997) Cell Calcium 22, 21–30.
[15] Noda, M., Yasuda-Fukazawa, C., Moriishi, K., Kato, T., Okuda, T., Kurokawa, K. and Takuwa, Y. (1995) FEBS Lett. 367, 246–250.
[16] Buus, C.L., Aalkjær, Nilsson, H., Juul, B., Møller and Mulvany, M.J. (1998) J. Physiol. 510, 577–590.
[17] Trinkle-Mulcahy, L., Ichikawa, K., Hartshorne, D.J., Siegman, M.J. and Butler, T.M. (1995) J. Biol. Chem. 270, 18191–18194.
[18] Kimura, K., Ito, M., Amano, M., Chihara, K., Fukata, Y., Nakafuku, M., Yamamori, B., Feng, J., Nakano, T., Okawa, K., Iwamatsu, A. and Kaibuchi, K. (1996) Science 273, 245–248.
[19] Eto, M., Ohmori, T., Suzuki, M., Furuya, K. and Morita, F. (1995) J. Biochem. 118, 1104–1107.
[20] Li, L., Eto, M., Lee, M.R., Morita, F., Yazawa, M. and Kitazawa, T. (1998) J. Physiol. 508, 871–881.
[21] Gong, M.C., Iizuka, K., Nixon, G., Browne, J.P., Hall, A., Eccleston, J.F., Sugai, M., Kobayashi, S., Somlyo, A.V. and Somlyo, A.P. (1996) Proc. Natl. Acad. Sci. USA 93, 1340–1345.
[22] Fujihara, H., Walker, L.A., Gong, M.C., Lemichez, E., Boquet, P., Somlyo, A.V. and Somlyo, A.P. (1997) Mol. Biol. Cell 8, 2437–2447.
[23] Kureishi, Y., Kobayashi, S., Amano, M., Kimura, K., Kanaide, H., Nakano, T., Kaibuchi, K. and Ito, M. (1997) J. Biol. Chem. 272, 12257–12260.
[24] Lucius, C., Arner, A., Steusloff, A., Troschka, M., Hofmann, F., Aktories, K. and Pfitzer, G. (1998) J. Physiol. 506, 83–93.
[25] Kitazawa, T., Kobayashi, S., Horiuti, K., Somlyo, A.V. and Somlyo, A.P. (1989) J. Biol. Chem. 264, 5339–5342.
[26] Kitazawa, T., Gaylinn, B.D., Denney, G.H. and Somlyo, A.P. (1991) J. Biol. Chem. 266, 1708–1715.
[27] Kobayashi, S., Kitazawa, T., Somlyo, A.V. and Somlyo, A.P. (1989) J. Biol. Chem. 264, 17997–18004.
[28] Mougios, V. and Bárány, M. (1986) Biochim. Biophys. Acta 872, 305–308.
[29] Gaylinn, B.D., Eddinger, T.J., Martino, P.A., Monical, P.L., Hunt, D.F. and Murphy, R.A. (1989) Am. J. Physiol. 257, C997–C1004.
[30] Himpens, B., Kitazawa, T. and Somlyo, A.P. (1990) Eur. J. Physiol. 417, 21–28.
[31] Itagaki, M., Komori, S., Unno, T., Syuto, B. and Ohashi, H. (1995) Jpn. J. Pharmacol. 67, 1–7.
[32] Nakanishi, H. and Exton, J.H. (1992) J. Biol. Chem. 267, 16347–16354.
[33] Sunyer, T., Codina, J. and Birnbaumer, L. (1984) J. Biol. Chem. 259, 15447–15451.
[34] Ichikawa, K., Ito, M. and Hartshorne, D.J. (1996) J. Biol. Chem. 271, 4733–4740.
[35] Chuang, T.-H., Bohl, B.P. and Bokoch, G.M. (1993) J. Biol. Chem. 268, 26206–26211.
[36] Hori, M., Sato, K., Miyamoto, S., Ozaki, H. and Karaki, H. (1993) Br. J. Pharmacol. 110, 1527–1531.
[37] Walker, L.A., Gailly, P., Jensen, P.E., Somlyo, A.V. and Somlyo, A.P. (1998) FASEB J. 12, 813–821.
[38] Iizuka, K., Yoshii, A., Dobashi, K., Nakazawa, T. and Mori, M. (1999) Biophys. J. (submitted).
[39] Somlyo, A.P. (1997) Nature (News Views) 389, 908–911.