Rho kinase inhibition partly weakens myogenic reactivity in rat small arteries by changing calcium sensitivity

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Schubert, Rudolf, Vjatscheslav U. Kalentchuk, and Ulrike Krien. Rho kinase inhibition partly weakens myogenic reactivity in rat small arteries by changing calcium sensitivity. Am J Physiol Heart Circ Physiol 283: H2288–H2295, 2002. First published August 29, 2002; 10.1152/ajpheart.00549.2002.—The hypothesis that Rho kinase is involved in myogenic reactivity was investigated in pressurized rat tail small arteries using videomicroscopic diameter determination and calcium fluorimetry. The potent Rho kinase inhibitor Y-27632 reversibly increased vessel diameter at 80 mmHg without changing the intracellular calcium concentration ([Ca],) shifting the relationship between diameter change and [Ca], to higher calcium levels. Neither endothelium removal nor inhibition of neural transmission affected the Y-27632-induced effect. Y-27632 at 3 × 10-6 mol/l attenuated the myogenic response in the pressure range from 10 to 120 mmHg, shifting the relationship between vessel tone and [Ca], to higher calcium levels. In addition, the Y-27632-induced shift of the relationship between vessel tone and [Ca], was larger at 80 than at 10 mmHg. These results suggest that smooth muscle cell Rho kinase in rat tail small arteries 1) is in an active state partly determining the level of the myogenic tone, and 2) alters the strength of the myogenic response by changing calcium sensitivity, probably caused by the pressure-induced activation of the kinase.

myogenic response; smooth muscle; contractility

THE PROCESS OF MATCHING BLOOD FLOW to metabolic demand during changes in perfusion pressure is determined to a large extent by the myogenic properties of small arteries. Myogenic properties are represented by the basal myogenic tone and the myogenic response. The myogenic tone is the maintained constriction state of small arteries produced by a permanently applied constant transmural pressure. The myogenic response is characterized by the constriction of small arteries after increases of transmural pressure and the dilation of these vessels after decreases of transmural pressure.

In recent years, considerable effort had been focused on the investigation of the mechanisms of the myogenic response of small arteries (for review see Refs. 3 and 12). Briefly, at the cellular level the following chain of events seems to be involved in pressure-induced vessel reactions. A pressure increase leads to an opening of smooth muscle stretch-activated cation channels producing a membrane depolarization. The depolarization activates voltage-operated calcium channels causing an enhanced influx of extracellular calcium. Subsequently, activation of myosin light chain kinase by calcium augments LC20 phosphorylation, followed by vessel constriction. In addition, the membrane depolarization produces an activation of voltage-dependent potassium channels and, together with the calcium increase, an activation of calcium-activated potassium channels. The activated potassium channels evoke a membrane hyperpolarization limiting the pressure-induced constriction. In summary, the myogenic reactivity of small arteries is characterized by a balance of several contractile and relaxing mechanisms.

Recently, it has been suggested that a change of the sensitivity of the contractile apparatus for intracellular calcium may be involved in pressure-induced vessel reactions. Thus it was observed that increasing the size of pressure steps produced greater myogenic responses despite similar steady-state calcium levels in hamster cheek pouch arterioles (2). In addition, the pressure-induced constriction of rat mesenteric small arteries was accompanied only by a small increase of the intracellular calcium concentration together with a high calcium sensitivity of the contractile apparatus (17). Furthermore, it was proposed that PKC may be mediating pressure-induced changes in calcium sensitivity, because PKC-inhibitors have been shown to attenuate the myogenic response in some arteries (1, 9, 18, 19), and PKC-α translocation was observed on a pressure increase from 40 to 100 mmHg (4). Recently, a report (16) appeared showing that a Rho kinase inhibitor abolished myogenic reactivity, suggesting that Rho kinase may also be mediating pressure-induced changes in calcium sensitivity. In summary, an increasing number of reports supports the idea that a change of the sensitivity of the contractile apparatus for intracellular calcium may be involved in pressure-induced vessel reactions.

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The report proposing recently the involvement of Rho kinase in pressure-induced vessel reactions (16) has evoked several new questions. First, in that study, the Rho kinase inhibitor Y-27632 abolished completely the myogenic tone and the myogenic response in mesenteric small arteries. Another study (19) published by the same group suggested that PKC is also involved in pressure-induced reactions of the same vessel, the mesenteric small artery. However, the Rho kinase inhibitor Y-27632 has been reported to interact with PKC, albeit with lower affinity. Thus the effect of Y-27632 on the myogenic tone and the myogenic response in mesenteric small arteries may be partly due to an interaction with PKC. Hence, the degree of participation of Rho kinase in myogenic reactivity may be smaller than suggested by the complete removal of this reactivity by Y-27632. Second, the effect of the Rho kinase inhibitor Y-27632 on the myogenic tone and the myogenic response in mesenteric small arteries may be partly due to an interaction with PKC. Therefore, the aim of the present study was to test the degree of participation of Rho kinase in myogenic reactivity of small arteries and whether this participation is due to a pressure-induced activation of Rho kinase. In summary, a profound knowledge of the mechanisms of pressure-induced changes in calcium sensitivity, especially concerning Rho kinase, is still lacking.

METHODS

The methods used in this study will be described briefly because they have been presented in detail previously (5, 11).

Dissection and mounting of the vessels. Small tail arteries were obtained from male Wistar-Kyoto rats by procedures in accordance with institutional guidelines. These vessels are first-order branches of the main ventral tail artery running parallel along the main tail artery (Fig. 1). These vessels have been selected because of their marked myogenic response, which has been shown to be PKC independent in preliminary studies (see also RESULTS). They were mounted in the experimental chamber containing experimental solution (physiological saline solution, PSS) consisting of (in mmol/l) 120 NaCl, 4.5 KCl, 1.2 NaH2PO4, 1.0 MgSO4, 1.6 CaCl2, 0.025 EDTA, 5.5 glucose, 26 NaHCO3, and 5 HEPES at pH 7.4. The microscope image of the vessel was viewed with a CCD camera and digitized by a frame-grabber board. Diameter reactions were analyzed online.

Experimental protocol. The vessel was pressurized to 80 mmHg. Leaking vessels were discarded at any stage of the experiment to ensure complete nonflow conditions. The temperature was set to 37.0 ± 0.5°C, and the pH was set to 7.40 ± 0.05. A P02 of ~150 mmHg and a PC02 of ~40 mmHg of the PSS in the experimental chamber were measured. After a spontaneous myogenic tone had developed, vessel viability was tested with 10−6 mol/l acetylcholine and 10−7 mol/l norepinephrine. The desired experiments were then performed. In some experiments the endothelium was removed by passing air through the lumen of the vessel. In other experiments, neural transmission was inhibited by applying phenotolamine (for details see Ref. 11).

Determination of intracellular calcium. After the viability test, vessels were loaded with 5 × 10−6 mol/l fura 2-AM at 37°C for 50–80 min. During measurements, the dye was excited with light at 340 and 380 nm with use of a filter wheel and a xenon arc lamp. The emission from the vessel was filtered at 520 nm, detected by a photomultiplier, and sent to a computer. There, the program used for diameter measurements also calculated the ratio of emission at the two excitation wavelengths after subtraction of the background fluorescence and presented the emission signals and the ratio together with the vessel diameter on the monitor screen. Calibration of the ratio in terms of calcium was not performed because of the numerous uncertainties inherent to this method. Steady-state calcium values have been used for all presentations.

Drugs and chemicals. Norepinephrine, acetylcholine, ionomycin, as well as the salts for the solutions, were obtained from Sigma (Deisenhofen, Germany). Fura 2-AM was purchased from Molecular Probes (Leiden, The Netherlands); Ro 31-8220, bisindolylmaleimide I, PD-98059, and calyculin A were from Calbiochem (Bad Soden, Germany). The Rho kinase inhibitor Y-27632 was generously provided by Welldis (Osaka, Japan).

Statistics. All data are means ± SE; n is the number of vessels. Statistical analysis was performed using t-test, one-way ANOVA, or repeated-measures ANOVA, as appropriate (SPSS 9.0 for Windows).

RESULTS

Rat tail small arteries exposed to a constant transmural pressure of 80 mmHg developed a myogenic tone shown as a vessel diameter reduction to 72.0 ± 1.4% (n = 32) of the diameter of the fully relaxed state, which was determined in calcium-free solution.

Effect of inhibition of Rho kinase on the myogenic tone. Application of Y-27632, a potent inhibitor of Rho kinase (15), concentration-dependently weakened the myogenic tone at 80 mmHg. This effect was shown as a reversible increase of vessel diameter (n = 6; P < 0.001) characterized by a Pd2 of 5.74 and a maximum
reaching 36.1% of the fully relaxed state, where the latter was determined in calcium-free solution at the end of each experiment (Fig. 2, A and B).

The dilation of the isobaric preparation of the vessel induced by the Rho kinase inhibitor was not accompanied by a change of the intracellular calcium concentration. Thus Y-27632 was applied at a submaximal concentration of $3 \times 10^{-6}$ mol/l to ensure a selective action of the inhibitor on Rho kinase. This altered the intracellular calcium concentration by $+5.2 \pm 3.8\%$, which was not different from the $-2.2 \pm 2.5\%$ change during the time control ($n = 6; P = 0.24$) (Fig. 3A). In contrast, when a dilation similar to the one evoked by Y-27632 was produced by reduction of the extracellular calcium concentration, the intracellular calcium concentration was considerably decreased by $-39.5 \pm 3.4\%$ compared with the $-2.2 \pm 2.5\%$ change during time control ($n = 6, P < 0.001$) (Fig. 3A).

Because these data suggest that Y-27632 reduces the calcium sensitivity of the contractile apparatus, calcium sensitivity was studied in vessels bathed in 42 mmol/l K-PSS to facilitate the opening of voltage-operated calcium channels. This solution initially did not contain calcium. The latter was added in log increments from $3 \times 10^{-6}$ to $3 \times 10^{-3}$ mol/l either in the absence or in the continuous presence of $3 \times 10^{-6}$ mol/l Y-27632. Addition of calcium produced a reduction of vessel diameter, which was smaller in the presence than in the absence of Y-27632 ($n = 5; P < 0.001$), and produced an increase of the intracellular calcium concentration, which was not different in the presence and in the absence of Y-27632 ($n = 5; P = 0.19$) (Fig. 3B). As a consequence, the curve of the relationship between diameter change and the intracellular calcium concentration, obtained from the extracellular calcium versus diameter and the extracellular calcium versus intracellular calcium relationships, was shifted to higher calcium levels in the presence of Y-27632 (Fig. 3B).

Figure 3B shows that readdition of external calcium produced smaller contractions in the presence of Y-27632 compared with its absence up to a calcium concentration of 3 mmol/l, the highest concentration tested. However, the addition of $10^{-6}$ mol/l ionomycin in the presence of 3 mmol/l external calcium resulted in a large increase of the intracellular calcium concentration accompanied by a vessel contraction reaching $97.0 \pm 1.3\%$ ($n = 5$) of the contraction induced by 3 mmol/l calcium in the absence of Y-27632. This effect was not different from the $96.2 \pm 2.8\%$ ($n = 5$) contraction induced by 3 mmol/l calcium in a time-control experiment ($P = 0.82$). Furthermore, the vasodilation induced by Y-27632 was reversed by the subsequent addition of calyculin A, a phosphatase inhibitor. Thus addition of $10^{-7}$ mol/l calyculin A constricted vessels to

![Fig. 2](https://placeboimage.com) Effect of inhibition of Rho kinase on the myogenic tone of rat tail small arteries. A: original record of the effect of the Rho kinase inhibitor Y-27632 on vessel diameter. Application of Y-27632 concentration-dependently weakened the myogenic tone at 80 mmHg shown as a reversible increase of vessel diameter. B: concentration-response relationship of the effect of Y-27632 on vessel diameter demonstrating a significant action of the inhibitor ($n = 6; P < 0.001$).

![Fig. 3](https://placeboimage.com) Effect of inhibition of Rho kinase on intracellular calcium and calcium sensitivity at 80 mmHg. A: application of $3 \times 10^{-6}$ mol/l Y-27632 to vessels (bar labeled Y-27632) did not alter the intracellular calcium concentration ($[Ca^{2+}]_i$) compared with application of physiological saline solution (PSS) (time control) ($n = 6; P = 0.24$). In contrast, reduction of the extracellular calcium concentration, producing a similar dilation as the one evoked by Y-27632 (102.5 ± 9.5% of the Y-27632-induced dilation) reduced the $[Ca^{2+}]_i$, compared with application of PSS ($n = 6, P < 0.001$). B: relationship between $[Ca]_i$ and diameter changes obtained by adding extracellular calcium in log increments from $3 \times 10^{-6}$ mol/l to $3 \times 10^{-3}$ mol/l. $[Ca^{2+}]_i$ is normalized to the corresponding data obtained during a single test application of $3 \times 10^{-3}$ mol/l extracellular calcium in 42 mmol/l K-PSS to reduce vessel to vessel variability. There was a difference in diameter change ($n = 5; P < 0.001$) but no significant difference in the $[Ca^{2+}]_i$ ($n = 5; P = 0.19$) in the presence of Y-27632.

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a level 10.9 ± 3.9% (n = 4) larger than the initial tone, i.e., the tone the vessel had before the dilation induced by Y-27632. This effect was not different from the 1.0 ± 1.3% (n = 4) change of tone during time control (P = 0.18).

Functional localization of Rho kinase. Application of Y-27632 to endothelium-denuded vessels weakened the myogenic tone at 80 mmHg. Here, Y-27632 at 3 × 10⁻⁶ mol/l increased vessel diameter by 26.0 ± 3.4% (n = 5), which was not different from the 25.9 ± 4.3% (n = 5) increase of vessel diameter observed in endothelium-intact vessels (P = 0.99). Application of Y-27632 to vessels with blocked transmission from nerve endings also weakened the myogenic tone at 80 mmHg. Here, Y-27632 at 3 × 10⁻⁶ mol/l increased vessel diameter by 26.5 ± 5.7% (n = 5), which was not different from the 25.9 ± 4.3% (n = 5) increase of vessel diameter observed in nonblocked vessels (P = 0.94).

Effect of inhibition of Rho kinase on the myogenic response. Application of pressure steps produced typical myogenic responses: a transient increase in diameter followed by an active constriction (Fig. 4A). In the presence of 3 × 10⁻⁶ mol/l Y-27632, the myogenic response was attenuated as shown by reduced pressure-induced constrictions at all pressure steps (n = 8; P < 0.001) (Fig. 4, A–D). In contrast, myogenic reactivity was not changed after inhibition of the PKC or of the mitogen-activated protein (MAP) kinase pathway. These mechanisms previously had been reported to be able to alter calcium sensitivity and to be activated by pressure increases. Thus neither the myogenic response to a pressure step from 80 to 120 mmHg nor the myogenic tone at 80 mmHg were affected by inhibition of the PKC pathway with two different inhibitors, 10⁻⁶ mol/l Ro 31-8220 and 10⁻⁷ mol/l bisindolylmaleimide I, or of the MAP kinase pathway with 10⁻⁵ mol/l PD-98059 (Table 1).

The pressure-induced constrictions were accompanied by increases of the intracellular calcium concentration. Higher levels of the intracellular calcium concentration were obtained in the presence compared with the absence of 3 × 10⁻⁶ mol/l Y-27632 (n = 7; P < 0.05) (Fig. 5, A and B). Smaller pressure-induced constrictions at higher levels of the intracellular calcium concentration in the presence of Y-27632 suggest that Y-27632 reduces the calcium sensitivity of the contractile apparatus. However, an analysis of vessel reactions at different pressure levels should take into account that pressure induces not only constriction but

**Table 1. Effect of inhibition of protein kinase C and mitogen-activated protein kinase pathway on myogenic tone and myogenic response**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>At 80 mmHg</th>
<th>At pressure step from 80 to 120 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−0.7 ± 0.1</td>
<td>−8.8 ± 2.5</td>
</tr>
<tr>
<td>Ro 31-8220 (10⁻⁶ mol/l)</td>
<td>−0.8 ± 0.9</td>
<td>−7.9 ± 2.2</td>
</tr>
<tr>
<td>Control</td>
<td>−0.2 ± 0.7</td>
<td>−7.2 ± 0.8</td>
</tr>
<tr>
<td>Bisindolylmaleimide I (10⁻⁷ mol/l)</td>
<td>−1.7 ± 0.6</td>
<td>−6.5 ± 2.2</td>
</tr>
<tr>
<td>Control</td>
<td>−0.1 ± 0.4</td>
<td>−10.1 ± 2.8</td>
</tr>
<tr>
<td>PD-98059 (10⁻⁵ mol/l)</td>
<td>−0.1 ± 0.5</td>
<td>−10.2 ± 2.6</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 vessels for all group. Significant differences were not observed.
also causes passive distention of the vessel. Therefore, the effect of Y-27632 on the calcium sensitivity of the contractile apparatus at different pressure levels was assessed by calculating vessel tone. Vessel tone is a measure of the state of activation taking into account the passive and the maximal active diameter-pressure relations (for details, see Ref. 17). Pressure increases produced an augmentation of vessel tone, which was not different in the presence and in the absence of Y-27632 ($n=7; P<0.29$). However, significantly higher levels of the intracellular calcium concentration were obtained in the presence of $3 \times 10^{-6} \text{mol/l}$ Y-27632 as described earlier (Fig. 5C). As a consequence, the curve of the relationship between tone and the intracellular calcium concentration, obtained from the pressure versus diameter and the pressure versus intracellular calcium relationships, was shifted to higher calcium levels in the presence of Y-27632 (Fig. 5C).

**Estimation of the activity of Rho kinase at different pressure levels.** The effect of the Rho kinase inhibitor Y-27632 on the myogenic response may reflect either a reduction of a constitutive, pressure-independent activity of Rho kinase or an inhibition of a pressure-induced activation of Rho kinase. To discriminate between these two mechanisms, Rho kinase activity was estimated by determining the Y-27632-sensitive part of the calcium sensitivity of the contractile apparatus at two different pressure levels, 10 and 80 mmHg. Calcium sensitivity was studied by adding calcium to vessels bathed in 42 mmol/l K-PSS as described in Effect of inhibition of Rho kinase on the myogenic tone. The curve of the relationship between tone and the intracellular calcium concentration obtained from the extracellular calcium versus diameter and the extracellular calcium versus intracellular calcium relationships was shifted to lower calcium levels at 80 mmHg compared with 10 mmHg (Fig. 6). Furthermore, the Y-27632-induced shift of the curve of the relationship between tone and the intracellular calcium concentration to higher calcium levels was larger at 80 mmHg than at 10 mmHg (Fig. 6). Thus the Rho kinase inhibitor shifted the calcium-tone relationship by $0.37 \pm 0.07$ ($n=5$) units of normalized calcium at 80 mmHg, compared with $0.12 \pm 0.04$ ($n=5$) units of normalized calcium at 10 mmHg ($P<0.05$ at a tone level of 0.023).
which was different from the shift of 0.12 ± 0.04 (n = 5) units of normalized calcium at 10 mmHg (P < 0.05). To differentiate stretch-activated Rho kinase from myogenic-related Rho kinase activation, the same experimental procedure was repeated using an isobaric preparation of the large main tail artery (for orientation see Fig. 1). This vessel does not show a myogenic tone or a myogenic response in the pressure range from 10 to 120 mmHg. It was observed that in the large tail artery the curve of the relationship between tone and the intracellular calcium concentration obtained from the extracellular calcium versus diameter and the extracellular calcium versus intracellular calcium relationships were shifted to lower calcium levels at 80 mmHg compared with 10 mmHg. However, in contrast with the small tail artery, the Y-27632-induced shift of the curve of the relationship between tone and the intracellular calcium concentration to higher calcium levels was similar at 80 and 10 mmHg. Thus the Rho kinase inhibitor shifted the calcium-tone relationship by 0.37 ± 0.05 (n = 4) units of normalized calcium at 80 mmHg, which was not significantly different from the shift of 0.40 ± 0.10 (n = 4) units of normalized calcium at 10 mmHg (P = 0.82).

DISCUSSION

Effect of Y-27632 on the myogenic tone and the myogenic response. In the present study, the novel finding is that the Rho kinase inhibitor Y-27632 only partly reduced the myogenic tone of an in vitro preparation of rat small arteries with a pD2 of 5.74 and only partly attenuated the myogenic response. In contrast to these findings, a recent study showed that Y-27632 completely abolished myogenic tone and the myogenic response in rat mesenteric small arteries, although the potency of Y-27632 (pD2 value not reported) was comparable to the present study (16). This discrepancy may be due to differences in the degree of participation of Rho kinase in myogenic reactivity in different vessels or be due to an interaction of the Rho kinase inhibitor Y-27632 with PKC in mesenteric small arteries, so that the degree of participation of Rho kinase in myogenic reactivity in these arteries is smaller than suggested by the complete removal of this reactivity by Y-27632.

The myogenic tone and the myogenic response are determined to a large extent by the level of the intracellular calcium concentration. However, the Y-27632-induced reduction of the myogenic tone observed in the present study was not accompanied by a decrease of the intracellular calcium concentration. Rather, the calcium concentration was stable during the tone reduction following application of Y-27632. In contrast to the effect of Y-27632, a dilation produced by reduction of the extracellular calcium concentration, which evoked a similar degree of dilation as Y-27632, was accompanied by a considerable decrease of the intracellular calcium concentration. Thus the vessel was in a functional state, where mechanisms producing vasodilation by a decrease of the intracellular calcium concentration could have been identified. Furthermore, the Y-27632-induced attenuation of the myogenic response observed in the present study was not accompanied by reduced levels of the intracellular calcium concentration. In contrast, the calcium concentrations at the higher pressure levels were increased in the presence of Y-27632. This phenomenon probably has nothing to do with a direct action of Y-27632 but can easily be explained by the higher wall tension at the larger vessel diameters in the presence of Y-27632. Higher levels of wall tension have been shown to be correlated with higher intracellular calcium concentration during myogenic responses (21). In the literature, vessel dilations or reduced contractions not accompanied by a decreased calcium concentration have been attributed to a reduction of the calcium sensitivity of the contractile apparatus as a consequence of down-regulation of myosin light chain kinase and/or upregulation of myosin light chain phosphatase (SMPP-1M), i.e., to desensitization to calcium (for review see Ref. 13). Indeed, the present study shows that the curve of the relationships between diameter change and the intracellular calcium concentration as well as between vessel tone and the intracellular calcium concentration were shifted to higher calcium levels in the presence of Y-27632. In summary, the data of the present study show that the reduction of the myogenic tone and the attenuation of the myogenic response evoked by Y-27632 are caused by a decrease in calcium sensitivity.

Selectivity of the effect of Y-27632 on the myogenic tone and the myogenic response. The Y-27632-induced change in calcium sensitivity observed in the present study is in accordance with the reported potent interaction of Y-27632 with Rho kinase (15). Y-27632 did not directly affect the intracellular calcium concentration, suggesting that Y-27632 does not interact with mechanisms responsible for calcium influx and efflux or calcium store release and refilling. The same conclusion was reached in a recent study on rat mesenteric small arteries (16). Concerning the mechanisms known to alter calcium sensitivity, four observations have been made in the present study. First, the Y-27632-induced reduction of contractile reactions could be overcome by an increase of intracellular calcium produced with use of ionomycin; the effect of Y-27632 was represented by a shift of the relationship between diameter change and the intracellular calcium concentration toward higher calcium values without reducing maximal contraction. This means that in the presence of high concentrations of Y-27632, i.e., after a marked inhibition of Rho kinase activity, these vessels still have a contractile apparatus capable of producing considerable constriction, albeit calcium concentrations higher than those reached during usual contractile reactions are required. Thus the effect of Y-27632 is not due to an unspecific loss of contractility. Second, it has been shown that the affinity of Y-27632 to PKC, a mechanism known to alter calcium sensitivity and to be involved in pressure-induced reactions, is 10 to 50 times lower compared with Rho kinase (15). The data
of the present study show that inhibitors of PKC, the kinase with the closest Y-27632-affinity compared with Rho kinase, do not affect the myogenic tone or myogenic response. Thus, even if PKC was inhibited partly by Y-27632 in the present study, this effect cannot explain the observed effect of Y-27632 on pressure-induced tone and the myogenic response. Third, it has been shown that the affinity of Y-27632 to myosin light chain kinase, a mechanism known to be involved in pressure-induced reactions, is >2,000 times lower compared with Rho kinase (15). The Y-27632-induced dilation observed in the present study was reversed by subsequent addition of calyculin A, a phosphatase inhibitor. This means that after addition of the highest Y-27632-concentration used, myosin phosphatase was active; an observation in accordance with the action of Y-27632 as a blocker of the myosin phosphatase inhibitor Rho kinase. In addition, the constriction observed after myosin phosphatase inhibition requires an active myosin light chain kinase. Thus these data indicate that inhibition of myosin light chain kinase is not a major mechanism involved in the effect of Y-27632. Finally, the data of the present study show that an inhibitor of the MAP kinase pathway, which has recently been shown to be activated by pressure increases (6, 8), does not affect the myogenic tone or the myogenic response. This conclusion is in accordance with a study on rabbit facial vein (6). Furthermore, the observation that neither endothelium removal nor inhibition of neural transmission were able to alter the response to Y-27632 shows that Y-27632 interacts with Rho kinase in smooth muscle cells. In summary, the data of the literature and of the present study suggest that the change of calcium sensitivity induced by Y-27632 is caused by a selective inhibition of smooth muscle cell Rho kinase.

Recently, another compound, HA-1077, has been reported to be a potent, concentration-selective inhibitor of Rho kinase (10). However, because HA-1077 has also been reported to interact with L-type calcium channels (7, 14), the use of this compound in functional experiments on the myogenic response was considered not useful.

Effect of a pressure increase on Rho kinase activity. The effect of the Rho kinase inhibitor Y-27632 on the myogenic response may reflect either a reduction of a constitutive, pressure-independent activity of Rho kinase or an inhibition of a pressure-induced change in the activity of Rho kinase. For an estimation of the activity of Rho kinase at different pressure levels in an in vitro artery preparation, in the present study it was considered that the activity of Rho kinase is reflected in the sensitivity of the contractile apparatus for calcium. The idea was, that if a pressure increase enhances the activity of Rho kinase, then the calcium sensitivity should be higher at higher pressure levels. In addition, the Rho kinase inhibitor Y-27632 should have a larger effect on calcium sensitivity at higher pressure levels. Indeed, the Y-27632-induced shift of the curve of the relationship between tone and the intracellular calcium concentration to higher calcium levels was larger at 80 than at 10 mmHg in the small tail artery. Furthermore, the finding that in the myogenically inactive large tail artery the Y-27632-induced shift of the curve of the relationship between tone and the intracellular calcium concentration to higher calcium levels was similar at 80 and at 10 mmHg indicates that the pressure-induced activation of Rho kinase is not a stretch- but a myogenic-related event. In this study, the question of a pressure-induced activation of Rho kinase was investigated using a functional approach. This approach was preferred because of 1) the great technical difficulties of a biochemical determination of Rho kinase activity in such extremely small vessel pieces with a diameter of ~200 μm and a length of not more than 400 μm, and of 2) the limited value, in addition to the technical difficulties mentioned above, of the determination of rhoA-translocation, because not only rhoA but also other compounds like arachidonic acid can activate Rho kinase. However, in a larger artery an isometric stretch-induced translocation of rhoA from the cytosol to the cell membrane has been reported (20). In summary, the data of the present study are consistent with the idea that a pressure increase can enhance the activity of Rho kinase in myogenically active arteries.

Role of Rho kinase for myogenic reactivity. The Y-27632-induced dilation and attenuation of pressure-induced constriction suggest that smooth muscle cell Rho kinase in rat tail small arteries is present and in an active state. Thus blood flow regulation can now be achieved with a variety of agonists not only by activating but also by inhibiting Rho kinase. The presented data show that the level of the myogenic tone and the strength of the myogenic response are not only determined by mechanisms regulating the intracellular calcium concentration but also by a mechanism altering the sensitivity of the contractile proteins for calcium, supporting the view that a change of the sensitivity of the contractile apparatus for intracellular calcium may be involved in pressure-induced vessel reactions (2, 16, 17, 19).

In conclusion, this study on rat tail small arteries presents the novel observation that Y-27632 only partly reduced the myogenic tone of an in vitro preparation of rat small arteries and only partly attenuated pressure-induced constrictions of these vessels. The reduction of the myogenic tone and the attenuation of the myogenic response evoked by Y-27632 is caused by a decrease of calcium sensitivity, most likely mediated by a change of Rho kinase activity. In addition, the data of this study suggest for the first time that a pressure increase can enhance the activity of Rho kinase. Thus, in the vessel investigated in the present study, Rho kinase is present and in an active state participating in blood flow regulation.
REFERENCES