RhoA/Rho kinase and nitric oxide modulate the agonist-induced pulmonary artery diameter response time

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Received 9 February 2001; accepted in final form 1 November 2001

Boer, Christa, Peter J. W. van der Linden, Gert Jan Scheffer, Nico Westerhof, Jaap J. de Lange, and Pieter Sipkema. RhoA/Rho kinase and nitric oxide modulate the agonist-induced pulmonary artery diameter response time. Am J Physiol Heart Circ Physiol 282: H990–H998, 2002. First published November 8, 2001; 10.1152/ajpheart.00093.2001.—We studied the amplitude and response time (RT; time to 50% of maximal response) of pulmonary vasoreactivity and investigated whether the characteristics of pulmonary vasoreactivity could be modulated by endothelium removal, nitric oxide (NO) synthase inhibition [Nω-nitro-L-arginine (L-NNA)], RhoA activation [lysophosphatidic acid (LPA)] and Rho kinase inhibition (Y-27632). Slow acetylcholine-induced pulmonary vasodilation (262 ± 5 s) was not due to the RT of endothelial NO release (45–55 s) and was always longer than RT in renal arteries (15 ± 4 s). The rate-determining step is located in the smooth muscle cells. This was confirmed by the existing differences between the RT of the NO solution and KCl-induced renal and pulmonary vasoreactivity in endothelium-denuded arteries. We found that the pulmonary contractile amplitude increases and the RT decreases by L-NNA or LPA. In contrast, Y-27632 reduced the contractile amplitude and increased the RT in pulmonary arteries. These phenomena were dependent on the contractile stimulus (phenylephrine or KCl). In conclusion, slow pulmonary vasoreactivity is a smooth muscle cell characteristic that can be enhanced by RhoA and NO or endothelium removal. These effects were counteracted by Rho kinase inhibition. We show a role for RhoA/Rho kinase and NO in the modulation of pulmonary vascular reactivity.

nitrergic nerve; endothelium; amplitude of constriction; response time

ISOLATED SYSTEMIC ARTERIES show large differences in the amplitude and speed of their responses to constrictors and dilators. These differences depend on the type and location of the vessel and differences in smooth muscle cell (SMC) characteristics (3, 9, 17, 18, 22). The characteristics of the vascular reactivity of pulmonary arteries, which form a special group in the circulation, are not well described in the literature.

The two objectives of the present study were therefore as follows: First, do pulmonary arteries exhibit similar magnitudes of contraction and response times to agonists as renal arteries? Renal arteries are, like pulmonary arteries, organ conduit arteries that are sensitive to similar agonists as pulmonary arteries. Second, if the magnitudes of contraction and response times are different, is it possible to modify these characteristics so that the renal and pulmonary vascular responses become similar?

With respect to the second objective, we investigated whether modification of the pulmonary vascular contractility and response time can be accomplished by changes in the sensitivity of the myosin light chain (MLC) for Ca2+. One of the ways in which the sensitivity of the contractile apparatus for Ca2+ can be modified is via changing the activity of MLC phosphatase.

Figure 1 shows the role of MLC phosphatase in the regulation of SMC contraction. MLC phosphatase is inactivated by Rho kinase, which is activated by a GTP-bound active form of Rho (RhoA), thereby increasing the SMC Ca2+ sensitivity (6, 10). RhoA can be activated by the bioactive lipid lysophosphatidic acid (LPA) and inhibited by nitric oxide (NO) via a cGMP protein kinase, whereas Rho kinase can be inhibited by Y-27632 (13, 16, 18). Thus LPA and NO inhibition would both lead to increased RhoA activity and therefore increased Ca2+ sensitivity, suggesting a similarity in their effects. To answer the second aim of our study in more detail, we therefore investigated whether RhoA activation or NO inhibition induces similar changes in the characteristics of pulmonary vascular reactivity and whether Rho kinase inhibition accomplishes complementary effects.

MATERIAL AND METHODS

Experimental animals. The Institutional Animal Care and Use Committee approved the experimental procedures, which conformed to the guidelines of Vrije Universiteit (Amsterdam, The Netherlands). Male Wistar rats (Harlan; Zeist, The Netherlands) were housed under standard conditions. Rats of 304 ± 11 g body wt (n = 41) were anesthetized with pentobarbital sodium (Nembutal, 60 mg/kg ip, Sanofi Sante BV; Maassluis, The Netherlands) and the lungs and/or left kidney were removed. Lungs or kidneys were pinned in a
dissecting dish containing MOPS buffer (4°C, pH 7.4), which consisted of (in mM) 145 NaCl, 5 KCl, 2 CaCl₂, 1 MgSO₄, 1 NaH₂PO₄, 5 dextrose, 2 pyruvate (Sigma-Aldrich; Zwijndrecht, The Netherlands), 0.02 EDTA, and 3 MOPS (all components except for pyruvate were obtained from Merck; Darmstadt, Germany).

Preparation and diameter studies. For vessel diameter studies, one pair of arteries was dissected from either the lung (two first-order side branches of the main pulmonary artery) or the kidney (two first-order side branches of the renal artery). The renal and pulmonary arteries are functionally equal: they are both organ conduit arteries with a comparable diameter and can be stimulated with the same agonists. KCl-induced constriction and the effects of RhoA activity were studied in endothelium-denuded arteries. The endothelium was deliberately removed from the arterial lumen with a bolus of 1 ml air, and the adequacy of endothelial removal was checked with acetylcholine.

For diameter measurements, one isolated artery was mounted in a pressure myograph consisting of a vessel chamber (bath volume, 2.5 ml) containing two cannulas, a circular heating coil, and a thermistor. The vessel chamber was filled with MOPS buffer (37°C, pH 7.4). The proximal and distal end of the artery were mounted onto the cannulas and secured with nylon sutures. Cannulas were chosen to fit vessel size. The proximal cannula was connected to a column filled with MOPS buffer, and the distal cannula was closed, i.e., there was no luminal flow. The applied transmural pressure was set at values appropriate for the two vessel types, 15 and 80 mmHg in pulmonary and renal arteries, respectively. Agonists were added to the buffer solution, which was superimposed by a pump (pump flow rate, 50 ml/h, Perfusor Secura B; Braun, Germany). Diameter changes were recorded with a video micrometer system camera (Sony Hyper Had SSC-DK38SP; Breda, The Netherlands) mounted on a microscope (Zeiss Stereomicroscope SV-6; Weesp, The Netherlands). The video camera was connected to an electronic measurement system to monitor the vessel diameter continuously.

NO measurements. For NO measurements, the second artery was cut open longitudinally and pinned on a silicon layer in a second organ bath. This organ bath was also filled with MOPS buffer, and agonists were added to the buffer and mixed. NO measurements were performed on the endothelial cell layer of the artery (21).

The electrochemical monitoring of NO was performed using a three-electrode system consisting of a NO microsensor, reference electrode, and counter electrode (sensitivity ±1 nM/pA). The NO microsensor was produced by threading a single carbon fiber (Ten Cate Advanced Composites; Amsterdam, The Netherlands) through a pulled end of a glass capillary, with a 2-mm length protruding according to a procedure previously described (12). The carbon fiber was coated with a polymeric layer of nickel (II) tetraks (3-methoxy-4-hydroxyphenyl) porphyrin (Interchim; Montluçon, France) and two 1.25% Nafion layers (Sigma-Aldrich). Potential differences were expressed between the coated fiber and the reference electrode (Ag-AgCl electrode). A platinum wire was used as counter electrode. The Electrochemical Microprobe System 100 (EMS-100, Biologic; Clax, France) was used for the differential normal pulsed amperometry. The oxidation potential for NO was determined from a voltamgram and was set to 680 mV during all amperometric experiments. With the use of a potential difference of 680 mV, the porphyrinic microsensor was free of interference from all reagents used in these experiments.

The sensitivity of each electrode (response time ~10 ms) was determined by calibration of the electrode after every experiment using aliquots of the NO solution. Aliquots of NO solution were prepared by serial dilutions of the saturated NO solution. To produce a saturated NO solution, deionized water was bubbled with pure argon (AGA Gas BV; Amsterdam, The Netherlands) for 20 min. This deoxygenated water was then bubbled with pure NO gas (AGA Gas BV) for 20 min and kept under a NO atmosphere until use. The saturated solution was diluted with deoxygenated water. The slope of the linear relation between the measured current (in pA) and the applied NO concentration (in nM) determined the sensitivity of the electrode.

Protocol. When an agonist was applied to the cannulated artery, the speed of the superfusion pump was increased transiently up to 1 ml/s (bath volume, 2.5 ml) to quickly replace the volume of buffer in the organ bath. For the first objective, we studied the amplitude and the response time of the diameter changes and NO release for acetylcholine, NO solution, and KCl. The response time was defined as the time interval to reach 50% of the maximal diameter change (RT₀.₅) or the maximal NO release (RT₀.₅,NO). First, the amplitude and response times of the acetylcholine-induced NO release and vasodilation were
studied in pulmonary and renal arteries. Arteries were constricted with l-phenylephrine hydrochloride (3 × 10⁻⁷ and 10⁻⁶ M phenylephrine in pulmonary and renal arteries, respectively, Sigma-Aldrich). NO release and vasodilation were studied in both artery types with increasing concentrations of acetylcholine, starting with 3 × 10⁻⁸ M until maximal vasodilation was induced (3 × 10⁻⁸–10⁻⁵ M, Sigma-Aldrich). The concentration of acetylcholine inducing maximal vasodilation was determined in separate experiments to avoid desensitization due to high concentrations of acetylcholine (10⁻⁶ M in renal and 10⁻⁵ M in pulmonary arteries, respectively). To check whether acetylcholine-induced vasodilation is completely NO mediated, NO release and diameter changes to 10⁻⁵ and 10⁻⁶ M acetylcholine (pulmonary and renal arteries, respectively) were also recorded after incubation of the vessels for 30 min with the NO synthase inhibitor N⁢-⁢G⁢-nitro-L-arginine (L-NNA; 10⁻⁴ M, Bachem; Bubendorf, Germany).

To study endothelium-independent vasodilation, subsequent concentrations of the NO solution were applied to endothelium-denuded pulmonary and renal arteries (3 × 10⁻⁹–3 × 10⁻⁷ M). NO solution was prepared as similar as the saturated NO solution used for the calibration of the NO electrodes.

Furthermore, the amplitude and RT₀.⁵,⁰ of KCl-induced constriction were compared in pulmonary and renal arteries that were both endothelium denuded. The high-KCl buffer consisted of (in mM) 90 NaCl, 60 KCl, 2 CaCl₂, 1 MgSO₄, 1 NaH₂PO₄, 5 dextrose, 2 pyruvate (Sigma-Aldrich), 0.02 EDTA, and 3 MOPS (all components except for pyruvate were obtained from Merck).

For the second objective, the amplitude and RT₀.⁵,⁰ of phenylephrine- or KCl-induced constriction in intact and endothelium-denuded pulmonary arteries were studied under control conditions and after incubation with the RhoA activator LPA (10⁻⁵ M, Sigma-Aldrich), the Rho kinase inhibitor Y-27632 (3 × 10⁻⁷ M, Tocris Cookson; Bristol, UK), and L-NNA (10⁻⁴ M). The effects of LPA and Y-27632 were studied in a random order in the same artery, which was exposed three times to phenylephrine or KCl. Papaverine (10⁻⁴ M, Sigma-Aldrich) was added to the superfusate to determine the maximal diameter.

Statistics and calculations. In all experiments, papaverine-induced vasodilation was used to determine the maximal passive diameters of pulmonary and renal arteries. Constrictor responses are expressed as the percent constriction from the maximal diameter. Vasodilatory changes are shown as the percent dilation during phenylephrine-induced constriction. For acetylcholine-induced NO measurements, the steady-state level of the NO response was taken as the measured concentration. Data are given in means ± SE. Statistical analysis was done by ANOVA and Bonferroni post hoc tests and paired or unpaired t-tests. Differences were considered statistically significant at P < 0.05.

RESULTS

Amplitude and response time of pulmonary and renal vasodilation. Starting diameters equaled maximal diameters obtained with papaverine and were 810 ± 27 and 1,178 ± 16 μm for renal and pulmonary arteries, respectively. Both pulmonary and renal arteries developed <5% spontaneous tone. Phenylephrine (3 × 10⁻⁷ and 10⁻⁶ M) induced 29.1 ± 0.8% and 20.4 ± 1.8% constriction in renal (n = 6) and pulmonary (n = 6) arteries, respectively. In phenylephrine-constricted arteries, a concentration-dependent acetylcholine-induced NO release and diameter change were measured (Fig. 2). Although 10⁻⁶ M acetylcholine induced more NO release in renal arteries (181 ± 75 nM) compared with pulmonary arteries (65 ± 10 nM), maximal NO release in both renal and pulmonary arteries was equal (175 ± 13 and 181 ± 75 nM for pulmonary and renal arteries, respectively). The highest acetylcholine-induced NO release also resulted in the largest diameter change (for 10⁻⁶ and 10⁻⁵ M of acetylcholine in renal and pulmonary arteries, respectively). After incubation with L-NNA, the acetylcholine-induced NO release was abolished in both vessel types (in renal and pulmonary arteries, P = 0.009 and P = 0.003, respectively; highest concentration acetylcholine vs. L-NNA, n = 6).

Renal and pulmonary arteries showed a vasodilation of 66 ± 6.5% and 70 ± 4.5%, respectively, for the highest concentration of acetylcholine. In both arteries, the addition of L-NNA diminished vasodilation (P = 0.006 and P = 0.004 in renal and pulmonary arteries, respectively; highest concentration acetylcholine vs. L-NNA). In summary, we found that the amplitude of the NO release and corresponding diameter were the same in renal and pulmonary arteries.

Fig. 2. Acetylcholine-induced changes in the amplitude of NO release and diameter in renal and pulmonary arteries. Addition of acetylcholine (ACh) induced a concentration-dependent increase in NO release in both vessel types that could be blocked with N⁢-⁢G⁢-nitro-L-arginine (L-NNA; A). Both renal and pulmonary arteries showed a similar maximal NO release on the highest concentrations of ACh (10⁻⁵ and 10⁻⁶ M, respectively). Increasing concentrations of ACh induce a concentration-dependent vasodilation in both preconstricted renal and pulmonary arteries (B). Incubation with L-NNA significantly prevented the ACh-induced vasodilation in both vessel types.
Figure 3 shows the response times of acetylcholine-induced NO release and vasodilation. As shown in Fig. 3A, renal and pulmonary arteries had the same RT_{0.5,NO} for acetylcholine-induced NO release in the range of 40–55 s, which was independent on the concentration of acetylcholine used. Thus (see Figs. 2A and 3A) we found that increasing concentrations of acetylcholine induce an increased NO release in both vessel types within the same time course.

Figure 3B shows that the acetylcholine-induced RT_{0.5,Ø} of both pulmonary and renal arteries was also acetylcholine concentration independent [not significant (NS)]. Because the signal-to-noise ratio for 10^{-7} M acetylcholine in renal arteries made determination of the RT_{0.5,Ø} unreliable, these data are not presented in Fig. 3. The RT_{0.5,Ø} was ~240 s for the pulmonary arteries and ~15 s for the renal arteries (P < 0.001, pulmonary vs. renal). In pulmonary arteries, the 10^{-8} M acetylcholine-induced RT_{0.5,NO} was 55 ± 5 s and the RT_{0.5,Ø} was 253 ± 5 s. In renal arteries, we found a 10^{-6} M acetylcholine-induced RT_{0.5,NO} of 45 ± 9 s and a RT_{0.5,Ø} of 15 ± 4 s, i.e., here the diameter response time is faster than the NO release.

Vascular reactivity characteristics in endothelium-denuded arteries. The slow pulmonary vascular reactivity, independent of the response time of NO release, suggests that the diameter response time is determined by smooth muscle cell characteristics. To study this in more depth, renal and pulmonary arteries were endothelium denuded to study NO solution-induced vasodilation in SMCs only (Fig. 4). NO solution induced a concentration-dependent vasodilation in both pulmonary and renal arteries that was slightly larger in renal than in pulmonary arteries (Fig. 4A). However, the RT_{0.5,Ø} of NO solution-induced vasodilation was larger in pulmonary arteries than in renal arteries (Fig. 4B). The RT_{0.5,Ø} of 3 × 10^{-7} M NO solution was 62.5 ± 14.3 s in pulmonary arteries (n = 6) and 16.0 ± 8.7 s in renal arteries (n = 6, P = 0.01). In general, NO solution-induced vasodilation was faster than acetylcholine-induced vasodilation in pulmonary arteries but not in renal arteries (Figs. 3 and 4).

Endothelium-denuded renal and pulmonary arteries were exposed to a vasoconstrictor to study whether the SMCs at this point account as well for possible differences in the RT_{0.5,Ø} between both artery types. The arteries were stimulated with KCl to exclude a role for differences in SMC membrane receptors between renal and pulmonary arteries.

The amplitudes and response times of KCl-induced vasoconstriction are shown in Fig. 5. The KCl-induced vasoconstriction was higher in renal arteries (44 ± 2%)
than in pulmonary arteries (23 ± 2%; \( P = 0.005 \), pulmonary vs. renal arteries; Fig. 5A). Figure 5B shows that the \( R_{0.5} \) of pulmonary arteries (106 ± 10 s) for KCI was about five times longer than the \( R_{0.5} \) of renal arteries (23 ± 5 s; \( P = 0.005 \), pulmonary vs. renal arteries; both \( n = 6 \)). In summary, these data suggest that the endothelium is not responsible for slow pulmonary vascular reactivity and that the slow pulmonary responses are present for both vasodilation (Figs. 3 and 4) and vasoconstriction (Fig. 5).

**Characteristics of pulmonary arteries can approach those of renal arteries after NO synthase inhibition.** In the second part of the study, we investigated whether slow pulmonary vascular reactivity can be modified such that the characteristics of pulmonary vascular reactivity resemble those of renal vasoreactivity. The \( \alpha_1 \)-adrenergic vasoconstrictor phenylephrine was used to study receptor-mediated constriction, whereas receptor-independent constriction was studied with KCl. Figure 6 represents the phenylephrine- or KCl-induced vasoconstriction in control arteries, endothelium-denuded arteries, and arteries that were incubated with L-NNA. Phenylephrine (3 \( \times \) 10\(^{-7}\) M) induced a vasoconstriction of 19.1 ± 1.7% in control arteries (Fig. 6A). The magnitude of constriction was increased by endothelium removal (28.8 ± 1.7%, \( P = 0.01 \) vs. control) or incubation with L-NNA (32.8 ± 2.2%, \( P = 0.03 \) vs. control, \( n = 7 \)).

![Figure 5](image1.png)

**Fig. 5.** The amplitude and \( R_{0.5} \) of KCl-induced vasoconstriction in endothelium-denuded renal (RA) and pulmonary arteries (PA). The amplitude of KCl-induced constriction was larger in endothelium-denuded renal than in pulmonary arteries (*\( P = 0.005 \), pulmonary vs. renal arteries; A). Furthermore, pulmonary arteries responded slower on KCl-induced constriction than renal arteries (*\( P = 0.005 \), pulmonary vs. renal arteries; B).

![Figure 6](image2.png)

**Fig. 6.** The effects of endothelium removal and L-NNA treatment on the amplitude and response time of phenylephrine (PE)- and KCl (K)-induced constriction in pulmonary arteries. A and B: effects of endothelium removal (−EC) and L-NNA treatment on the amplitude of PE- and KCl-induced constriction in pulmonary arteries. Endothelium removal and L-NNA treatment attenuated only the amplitude of PE-induced constriction. Endothelium removal and L-NNA treatment decreased the response times of both PE (C) and KCl (D). *\( P < 0.05 \) vs. control.
Figure 6B depicts the KCl-induced vasoconstriction for the same three conditions. Endothelium removal or L-NNA treatment did not change the contractile response of pulmonary arteries to KCl (23 ± 4% and 28 ± 3% for endothelium removal and L-NNA treatment, respectively) compared with the control situation (27 ± 4%).

Figure 6, C and D, represents the RT0.5,Ø of phenylephrine- and KCl-induced vasoconstriction in control, endothelium-denuded, and L-NNA-exposed pulmonary arteries. Figure 6C shows that the RT0.5,Ø for phenylephrine-induced constriction (63.1 ± 14.7 s) was reduced by both endothelium removal (27.5 ± 5.3 s, P < 0.01) and exposure to L-NNA (40.3 ± 4.2 s, P = 0.01). Similar results were obtained for KCl-induced constriction (Fig. 6D). The response times of KCl-induced constriction were larger than for phenylephrine-induced constriction.

Modulation of RhoA or Rho kinase changes the response time of pulmonary arteries. Figure 7 represents the effects of LPA and Y-27632 on pulmonary vascular reactivity in endothelium-denuded arteries. Figure 7A shows the effects of RhoA and Rho kinase activity changes on the magnitude of phenylephrine-induced constriction. LPA did not increase the amplitude of constriction, but application of Y-27632 decreased the level of constriction (8.8 ± 1.6%) compared with control arteries (28.8 ± 1.7%, P = 0.001). The magnitude of the KCl-induced contractile response was not affected by LPA or Y-27632 (Fig. 7B).

DISCUSSION

We found that the acetylcholine-induced diameter response time of pulmonary arteries is longer than that
of renal arteries. Slow acetylcholine-induced pulmonary vasodilation is not due to the response time of endothelial NO release, and the rate-determining step is located in SMCs. The latter was confirmed by the existing differences between the RT_{0.5,0} of NO solution-induced renal and pulmonary vasodilation in endothelium-denuded arteries. In addition, in renal arteries, the amplitude of KCl-induced vasoconstriction was higher with a lower response time than in pulmonary arteries.

Furthermore, we found that the amplitude of pulmonary artery constriction can be increased and the response time can be reduced by NO synthase inhibition or RhoA activation. In contrast, Rho kinase inhibition decreased the diameter amplitude and increased the response time of pulmonary arteries. We found that the RhoA pathway plays a major role in receptor-independent vasoconstrictors that act via different mechanisms. Phenylephrine activates phospholipase C and modulates the SMC Ca^{2+} concentration via the influx of Ca^{2+} and the release of Ca^{2+} from the sarcoplasmic reticulum (1, 11). In contrast, KCl-induced constriction acts mainly via direct depolarization of the SMC, resulting in Ca^{2+} influx via voltage-operated Ca^{2+} channels.

The amplitude of phenylephrine-induced vasoconstriction but not of KCl-induced vasoconstriction was increased after endothelium removal and NO inhibition. Pulmonary arteries maintain their low vascular resistance partly by the release of NO (14). Furthermore, it has been reported by our group (5) that phenylephrine induces NO release. The combination of basal and phenylephrine-induced NO release reduces the amplitude of phenylephrine-induced constriction. With the use of L-NNA, NO release is reduced and more constriction ensured via direct effects of cGMP on the SMC Ca^{2+} concentration or via an effect on the RhoA activity, concluding that the phenylephrine-induced constriction is an accumulation of vasoconstrictor and vasodilator effects. This suggests a role for endothelial NO release as a modulator of specific contractile responses in the pulmonary vasculature.

Although KCl might induce NO release as well via SMC Ca^{2+} influx via gap junctions, we found no effect of endothelium removal or NO synthase inhibition on the magnitude of KCl-induced constriction. These results suggest that the type of constriction also determines the characteristics of pulmonary vascular reactivity.

Both endothelium removal and L-NNA treatment diminished the response times of phenylephrine- and KCl-induced vasoconstriction. Remarkably, we were able to reduce the response time of the pulmonary contractile responses such that they were almost identical to those of the renal arteries. NO might be, like Y-27632, an inhibitor of the RhoA pathway and stimulate vasodilation. Removal of NO from the vasculature might result in RhoA activation and therefore an increased contractile amplitude and faster constriction. Furthermore, our results for KCl suggest that the first minutes in which the KCl-induced constriction develops, NO still diminishes the speed at which depolarization induces a SMC Ca^{2+} increase. Inhibition of NO will therefore result in an increased speed of constriction but also in an unaltered amplitude. These findings also suggest a more important effect of NO on the SMC Ca^{2+} concentration than on the Ca^{2+} sensitivity. For all experiments in pulmonary arteries, the RT_{0.5,0} of phenylephrine-induced constriction was lower than the RT_{0.5,0} of KCl-induced constriction. The pathway of Ca^{2+} increase via influx or the sarcoplasmic reticulum in combination with Ca^{2+} sensitivity is probably a

Effects of endothelium removal and NO synthase inhibition on pulmonary vascular reactivity. We subsequently investigated whether it is possible to modify pulmonary SMC characteristics via changing the MLC phosphatase activity. We used receptor-mediated and receptor-independent vasoconstrictors that act via different mechanisms. Phenylephrine activates phospholipase C and modulates the SMC Ca^{2+} concentration via the influx of Ca^{2+} and the release of Ca^{2+} from the sarcoplasmic reticulum (1, 11). In contrast, KCl-induced constriction acts mainly via direct depolarization of the SMC, resulting in Ca^{2+} influx via voltage-operated Ca^{2+} channels.

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faster process than depolarization-induced Ca$^{2+}$ influx. The KCl-induced intracellular Ca$^{2+}$ increase probably inhibits the sarcoplasmic Ca$^{2+}$ influx, thereby resulting in a slower contractile response compared with phenylephrine. For KCl-induced constriction, renal arteries were faster than pulmonary arteries. This difference is not due to a variance in the L-type voltage-operated Ca$^{2+}$ channels or Ca$^{2+}$ influx characteristics of both vessel types (2).

RhoA/Rho kinase are involved in the modulation of pulmonary vascular reactivity. The amplitudes of both phenylephrine- and KCl-induced constriction were not affected by LPA in endothelium-denuded pulmonary arteries. Others (19, 20) have confirmed these results for KCl but found a LPA-induced augmentation of the amplitude when constriction was induced by receptor-dependent agonists. However, in those studies, phenylephrine was not included. The absence of an LPA-induced augmentation of the amplitude of constriction suggests that RhoA activity cannot be enhanced in these arteries or that the arteries were maximally constricted. In contrast, Rho kinase inhibition decreased the amplitude of the phenylephrine response but not of KCl-induced constriction. This suggests that the Rho pathway is only involved in pharmacomechanical constriction. Our results suggest that the level of RhoA activity in SMCs determines the characteristics of vasoconstriction.

Although LPA did not affect the level of constriction, the response times of phenylephrine-induced contraction were decreased by LPA. The mechanism underlying this effect of LPA probably involves a LPA-induced increase of the Ca$^{2+}$ sensitivity of the contractile apparatus, resulting in faster myosin-actin coupling. The response time of the Ca$^{2+}$ influx cannot account for the attenuation of the RT$0.5$, of phenylephrine-induced constriction, because phenylephrine induces a maximal pulmonary SMC Ca$^{2+}$ increase within 10 s (7).

LPA affected only the response times of phenylephrine-induced constriction in intact but not in endothelium-denuded pulmonary arteries. Removal of NO probably decreased the response time of phenylephrine-induced vasoconstriction so that LPA did not enlarge this phenomenon. LPA did not affect the response times of KCl-induced constriction, suggesting that the response time was already optimal or that KCl-induced constriction is not mediated via Rho kinase. However, application of the Rho kinase inhibitor Y-27632 sensitized the RhoA activity of both arterial stimuli in endothelium-denuded arteries. We conclude that the level of RhoA activity affects the amplitude and response time of vasoconstriction.

We conclude that the RT$0.5$, of pulmonary vasodilation and vasoconstriction is determined by SMCs and is in all cases longer than the RT$0.5$, of renal SMCs. NO inhibition enhanced the characteristics of pulmonary vasoconstriction so that they resembled those of renal arteries. We were able to show opposite effects of LPA and Y-27632 on pulmonary constriction characteristics. Finally, a decreased NO synthase or an increased RhoA activity might play a role in a disturbed pulmonary vascular responsiveness and might therefore contribute to the induction of pathophysiological processes.

This study was supported by AGA Linde Healthcare, The Netherlands.

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