Cyclic ADP-Ribose Is the Primary Trigger for Hypoxic Pulmonary Vasoconstriction in the Rat Lung In Situ

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Cyclic ADP-Ribose Is the Primary Trigger for Hypoxic Pulmonary Vasoconstriction in the Rat Lung In Situ

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Abstract—Hypoxic pulmonary vasoconstriction (HPV) is unique to pulmonary arteries, and it aids ventilation/perfusion matching. However, in diseases such as emphysema, HPV can promote hypoxic pulmonary hypertension. We recently showed that hypoxia constricts pulmonary arteries in part by increasing cyclic ADP-ribose (cADPR) accumulation in the smooth muscle and, thereby, Ca\(^{2+}\) release by ryanodine receptors. We now report on the role of cADPR in HPV in isolated rat pulmonary arteries and in the rat lung in situ. In isolated pulmonary arteries, the membrane-permeant cADPR antagonist, 8-bromo-cADPR, blocked sustained HPV by blocking Ca\(^{2+}\) release from smooth muscle ryanodine-sensitive stores in the sarcoplasmic reticulum. Most importantly, we showed that 8-bromo-cADPR blocks HPV induced by alveolar hypoxia in the ventilated rat lung in situ. Inhibition of HPV was achieved without affecting (1) constriction by membrane depolarization and voltage-gated Ca\(^{2+}\) influx, (2) the release (by hypoxia) of an endothelium-derived vasoconstrictor, or (3) endothelium-dependent vasoconstriction. Our findings suggest that HPV is both triggered and maintained by cADPR in the rat lung in situ. (Circ Res. 2001;89:77-83.)

Key Words: cADP-ribose ■ pulmonary artery ■ hypoxia

Since it was first described, hypoxic pulmonary vasoconstriction (HPV) has been recognized as the critical and distinguishing characteristic of pulmonary arteries; systemic arteries dilate in response to hypoxia. Physiologically, HPV contributes to ventilation-perfusion matching in the lung. However, when alveolar hypoxia is global, as it is in disease states such as emphysema and cystic fibrosis, it results in pulmonary hypertension and, eventually, right heart failure. In isolated pulmonary arteries, HPV is biphasic. A transient constriction (phase 1) is followed by slow tonic constriction (phase 2). It was thought that phase 1 was initiated by a reduction in membrane K\(^{+}\) conductance in the smooth muscle and voltage-gated Ca\(^{2+}\) influx. In addition, the primary mediator of phase 2 of HPV was thought to be an endothelium-derived vasoconstrictor. Contrary to this, we and others discovered that hypoxia promotes phases 1 and 2 by releasing Ca\(^{2+}\) from ryanodine-sensitive stores in the sarcoplasmic reticulum (SR) by a mechanism intrinsic to the smooth muscle.

Recently, we showed that the β-NAD\(^+\) metabolite cyclic ADP-ribose (cADPR) plays a role in this process. Thus, hypoxia increases cADPR accumulation and Ca\(^{2+}\) release from smooth muscle, leading to constriction in isolated rabbit pulmonary arteries.

In the present investigation, we demonstrate that HPV is triggered and maintained by cADPR in isolated rat pulmonary arteries and in the rat lung in situ.

Materials and Methods

Dissection
Male Wistar rats (250 to 350 g) were anesthetized with 4% enflurane and exsanguinated. The heart and lungs were removed and placed in chilled physiological saline solution A containing (in mmol/L): 118 NaCl, 4 KCl, 24 NaHCO\(_3\), 1 MgSO\(_4\), 1.2 NaH\(_2\)PO\(_4\), 2 CaCl\(_2\), and 5.56 glucose (pH 7.4). Pulmonary arteries were dissected free and used immediately.

Small Vessel Myography
Third-order branches of the pulmonary artery (internal diameter, 300 to 400 μm; 2 to 3 mm in length) were mounted on the jaws of a myograph (AM10, Cumhust Biological) using 50 μm tungsten wire. Initial tension was equivalent to 30 mm Hg, and the chamber (8 mL) was maintained at 37 ± 1°C. The technique, protocol, and theory have been described previously. The endothelium was removed by rubbing the intima with braided silk surgical thread. Endothelium removal was confirmed by the failure of 100 μmol/L acetylcholine to relax constriction by 1 μmol/L prostaglandin F\(_2\alpha\) (PGF\(_2\alpha\)).

Experimental Protocol
Arteries were constricted by high K\(^+\) (75 mmol/L) before and after each experiment to test the responsiveness and stability of the preparation and to give a standard response for comparative studies (9 ± 3 mN/mm; n = 42). Resting tension was taken to be zero. Experimental chambers were covered and bubbled (150 mL/min) with normoxic gas at 154 to 160 Torr (75% N\(_2\), 20% O\(_2\), and 5% CO\(_2\)). When required, we switched to hypoxic gas at 47 to 52 Torr (89% N\(_2\), 6% O\(_2\), and 5% CO\(_2\)) or 16 to 21 Torr (93% N\(_2\), 2% O\(_2\), and 5% CO\(_2\)). Gas was supplied by a gas-mixing flowmeter (Cameron Instruments). All drugs were applied to the bath directly. All
solutions were warmed to 37°C and bubbled with 5% CO₂ to maintain pH 7.4.

**Perfused Lung**

Male Wistar rats (250 to 350 g) were anesthetized by 4% enflurane, injected with 1000 IU of heparin intravenously, and killed by exsanguination (approved by Home Office). A polyethylene catheter was inserted into the pulmonary artery through the right ventricle and tied in place. Another catheter was inserted into the left atrium. The lung was perfused (0.06 mL · min⁻¹ · g⁻¹) with 10 mL of physiological saline solution B containing (in mmol/L): 118 NaCl, 4 KCl, 1.2 NaH₂PO₄, 1 MgSO₄, 24 NaHCO₃, 2 CaCl₂, and 5.56 glucose and 4% Ficoll (albumin substitute) at pH 7.4. Pulmonary vascular perfusion pressure was measured by a pressure transducer that was located on a side arm of the pulmonary arterial catheter. From the left atrial catheter, the venous outflow entered a reservoir and was recirculated. The lungs were inflated (by a tracheotomy) 30 times per minute with normoxic gas (75% N₂, 20% O₂, and 5% CO₂). When required, we switched to hypoxic gas containing either 6% O₂ (balanced with 89% N₂) or 2% O₂ (balanced with 98% N₂ and 5% CO₂). Gas was supplied by a gas-mixing flowmeter (Cameron Instruments). Maximum ventilation pressure was <13 cm H₂O.

**Drugs**

All compounds were from Sigma. 8-Bromo-cADPR and caffeine were dissolved in distilled water or physiological saline solution. Ryanodine was dissolved in DMSO. The minimum dilution of DMSO was 1:10,000, which had no effect on the arteries.

**Results**

**HPV Is Abolished by Ryanodine and Caffeine**

Isolated pulmonary artery rings constricted biphasically by hypoxia (16 to 21 Torr; Figure 1A). Phase 1 peaked after 3 to 5 minutes at 63±7% of the constriction by 75 mmol/L K⁺ (n=4), and it then declined back to a level above pretone. Phase 2 of HPV then developed to a maximum of 39±9% (n=4) after 40 minutes of hypoxia. Removal of the endothelium had no effect on phase 1 of HPV. However, during phase 2, the progressive rise in tension was lost (Figure 1B), leaving a maintained plateau constriction that measured 13±4% (n=4).

Whether the endothelium was present (Figure 1C) or absent (Figure 1D), preincubation with caffeine (10 mmol/L) and ryanodine (10 µmol/L) abolished the constriction by hypoxia (16 to 21 Torr). In contrast, in the presence of ryanodine, caffeine, and hypoxia, the constriction by 75 mmol/L K⁺ was similar to control (9±2 mN/mm in the presence of the endothelium and 10±2 mN/mm in its absence; n=4). Thus, hypoxia initiates and maintains acute HPV in isolated rat pulmonary arteries by triggering Ca²⁺ release from ryanodine-sensitive SR stores.

**8-Bromo-cADPR Blocks Phase 2 but Not Phase 1 of HPV**

The effect of a membrane-permeant cADPR antagonist, 8-bromo-cADPR, was different from that of caffeine or ryanodine. Figure 2A shows the constriction of an intact pulmonary artery ring by hypoxia (16 to 21 Torr). Phase 1 peaked at 62±6% and phase 2 measured 43±7% after 40 minutes (n=6). Figure 2B shows the response in the absence...
The phase 2 constriction was also reduced from 43±6% to 22±4%, respectively (n=4). Preincubation with 8-bromo-cADPR (300 μmol/L) had no effect on the phase 1 constriction by 47 to 52 Torr hypoxia, which measured 29±5% (n=4), and the phase 2 constriction was abolished as before (Figure 3).

Cyclopiazonic Acid Blocks Phase 1 but Not Phase 2 of HPV

The fact that phase 1 of HPV remained unaffected in the presence of 8-bromo-cADPR but was abolished by ryanodine and caffeine suggested the involvement of a mechanism independent of cADPR but dependent on ryanodine-sensitive SR Ca2+ stores. Therefore, we investigated the possibility that hypoxia triggered phase 1 of HPV by inhibiting SR Ca2+ ATPase activity, leading to an increase in the net SR Ca2+ efflux. We used the selective Ca2+ ATPase antagonist cyclopiazonic acid. Figure 4A shows constriction by hypoxia in an intact artery. Phase 1 peaked at 60±7% and phase 2 measured 39±4% after 40 minutes (n=6). Figure 4B shows constriction by hypoxia in an artery without the endothelium. Phase 1 peaked at 59±7% and the residual phase 2 plateau measured 14±4% after 40 minutes (n=6). Preincubation (10 minutes) with 10 μmol/L cyclopiazonic acid abolished phase 1 of HPV in the presence (Figure 4C) and absence (Figure 4D) of the endothelium (n=4).

In contrast to ryanodine and caffeine (Figure 1C) and to 8-bromo-cADPR (Figure 2C), cyclopiazonic acid had no effect on phase 2 of HPV. In intact arteries after 40 minutes of hypoxia, phase 2 measured 39±8% in the absence and 39±6% in the presence of 10 μmol/L cyclopiazonic acid (Figures 4A and 4C; n=4). In the absence of the endothelium, the plateau constriction measured 13±4% in the absence and 13±4% in the presence of 10 μmol/L cyclopiazonic acid (Figures 4B and 4D; n=4). Thus, inhibition of SR Ca2+ ATPase activity with cyclopiazonic acid has no effect on the induction or magnitude of phase 2 of HPV in pulmonary artery rings.

Figure 4. Cyclopiazonic acid blocks phase 1 but not phase 2 of HPV in isolated arteries. A, Response of an intact artery ring by hypoxia (16 to 21 Torr). B, Response of a de-endothelialized artery by hypoxia (16 to 21 Torr). C, Effect of hypoxia (16 to 21 Torr) in an intact artery ring after 10 minutes of preincubation with cyclopiazonic acid (10 μmol/L). D, Response of a de-endothelialized artery by hypoxia (16 to 21 Torr) in the presence of cyclopiazonic acid (10 μmol/L). The records in A and C and in B and D, respectively, were obtained from the same artery.
sure to alveolar hypoxia induced by 2% O₂ in the absence and cADPR (Figures 5B and 5C; n=4).

C, Change in perfusion pressure by alveolar hypoxia induced by 6% O₂ in the absence and then in the presence of 300 μmol/L 8-bromo-cADPR (10 minutes preincubation). B, Change in perfusion pressure by alveolar hypoxia induced by 2% O₂ in the rat lung with a threshold of 30 μmol/L, and complete reversal with 300 μmol/L 8-bromo-cADPR (n=4). Figure 6B shows that cumulative application of 8-bromo-cADPR (1 to 300 μmol/L) inhibited the increase in perfusion pressure by alveolar hypoxia (2% O₂) in the rat lung with a threshold of 30 μmol/L, and complete reversal with 300 μmol/L 8-bromo-cADPR. In contrast, after preincubation with 3 μmol/L 8-bromo-cADPR (Figure 7B) phase 1 measured 61±5% (compared with 63±5% in its absence) and phase 2 (40±4% in absence) was abolished (n=4).

In the perfused and ventilated rat lung, hypoxia (2% O₂) increased perfusion pressure from 7±1 mm Hg to

Concentration-Dependence and Selectivity of 8-Bromo-cADPR

Figure 6A shows the effect of cumulative application of 8-bromo-cADPR (1 to 100 μmol/L) on the plateau constriction by hypoxia (16 to 21 Torr) in an isolated pulmonary artery ring without the endothelium (ie, the constriction maintained by Ca²⁺ release from ryanodine-sensitive SR stores). The plateau constriction was inhibited with a threshold for inhibition of 3 μmol/L, and complete reversal was obtained with 100 μmol/L 8-bromo-cADPR (n=4). Figure 6B shows that cumulative application of 8-bromo-cADPR (1 to 300 μmol/L) inhibited the increase in perfusion pressure by alveolar hypoxia (2% O₂) in the rat lung with a threshold of 30 μmol/L, and complete reversal with 300 μmol/L 8-bromo-cADPR (n=4). Figure 6C shows the concentration-inhibition curves for each preparation. The IC₅₀ for inhibition of HPV in isolated arteries and in the rat lung was 30 μmol/L and 55 μmol/L, respectively.

Curiously, preincubation with 8-bromo-cADPR blocked the initiation of HPV in an all-or-none manner but with a clear threshold concentration. In intact pulmonary artery rings (Figure 7A), phase 1 of HPV measured 62±6% in the absence and 64±6% in the presence of 1 μmol/L 8-bromo-cADPR. Phase 2 measured 42±5% in the absence and 40±5% in the presence of 1 μmol/L 8-bromo-cADPR. In contrast, after preincubation with 3 μmol/L 8-bromo-cADPR (Figure 7B) phase 1 measured 61±5% (compared with 63±5% in its absence) and phase 2 (40±4% in absence) was abolished (n=4).

In the perfused and ventilated rat lung, hypoxia (2% O₂) increased perfusion pressure from 7±1 mm Hg to

8-Bromo-cADPR Inhibits HPV in the Rat Lung In Situ

From the findings described above, 3 components of HPV in isolated pulmonary arteries can be separated pharmacologically: the constriction mediated by inhibition of SR Ca²⁺ ATPase (component 1), by cADPR-dependent Ca²⁺ release from ryanodine-sensitive SR stores in the smooth muscle (component 2), and by an endothelium-derived vasoconstrictor (component 3). Because components 1 and 3 were insensitive to 8-bromo-cADPR, we were able to investigate the contribution of cADPR to HPV in the perfused and ventilated rat lung in situ. Figure 5A shows that perfusion pressure increased from 7±1 mm Hg (n=6) with alveolar normoxia (20% O₂) to 14±1 mm Hg (n=6) with alveolar hypoxia (2% O₂). On return to normoxia, the perfusion pressure declined to 8±1 mm Hg (n=4), after which a second exposure to alveolar hypoxia (2% O₂) increased the perfusion pressure to 15±1 mm Hg (n=6). The increase in perfusion pressure was dependent on the degree of alveolar hypoxia. Figures 5B and 5C, respectively, show that the perfusion pressure increased from 7±1 mm Hg to 14±1 mm Hg when the alveoli were supplied with 2% O₂, and from 7±1 mm Hg to 11±1 mm Hg with 6% O₂ (n=4). The increase in perfusion pressure with 2% and 6% O₂, respectively, was abolished by preincubation (10 minutes) with 300 μmol/L 8-bromo-cADPR (Figures 5B and 5C; n=4).

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Figure 5. 8-Bromo-cADPR blocks HPV in the perfused and ventilated rat lung in situ. A, Increase in perfusion pressure in the perfused and ventilated rat lung in situ in response to 2 exposures to alveolar hypoxia (2% O₂). B, Change in perfusion pressure to alveolar hypoxia induced by 2% O₂ in the absence and then in the presence of 300 μmol/L 8-bromo-cADPR (10 minutes preincubation). C, Change in perfusion pressure by alveolar hypoxia induced by 6% O₂ in the absence and then in the presence of 300 μmol/L 8-bromo-cADPR (10 minutes preincubation).

Figure 6. Concentration-dependent inhibition of HPV in isolated rat pulmonary arteries and in the rat lung in situ by 8-bromo-cADPR. A, Constriction by hypoxia (16 to 21 Torr) in an isolated rat pulmonary artery ring without the endothelium, and the effect of cumulative application of 8-bromo-cADPR (1 to 100 μmol/L) on the plateau phase of the constriction. B, Increase in perfusion pressure in the rat lung in situ in response to alveolar hypoxia (2% O₂) and the effect of cumulative application of 8-bromo-cADPR (1 to 300 μmol/L). C, Concentration-inhibition curves for inhibition by 8-bromo-cADPR of maintained HPV in isolated, de-endothelialized pulmonary artery rings and in the rat lung in situ. The curves show the line of best fit to a Hill equation. Data from the isolated arteries were best fit with an IC₅₀ of 30 μmol/L and a coefficient of 1.2. Data from the perfused lung were best fit with an IC₅₀ of 55 μmol/L and a coefficient of 1.5. The points represent the mean and the vertical bars represent the SE for at least 4 experiments.

8-Bromo-cADPR Inhibits HPV in the Rat Lung In Situ

From the findings described above, 3 components of HPV in isolated pulmonary arteries can be separated pharmacologically: the constriction mediated by inhibition of SR Ca²⁺ ATPase (component 1), by cADPR-dependent Ca²⁺ release from ryanodine-sensitive SR stores in the smooth muscle (component 2), and by an endothelium-derived vasoconstrictor (component 3). Because components 1 and 3 were insensitive to 8-bromo-cADPR, we were able to investigate the contribution of cADPR to HPV in the perfused and ventilated rat lung in situ. Figure 5A shows that perfusion pressure increased from 7±1 mm Hg (n=6) with alveolar normoxia (20% O₂) to 14±1 mm Hg (n=6) with alveolar hypoxia (2% O₂). On return to normoxia, the perfusion pressure declined to 8±1 mm Hg (n=4), after which a second exposure to alveolar hypoxia (2% O₂) increased the perfusion pressure to 15±1 mm Hg (n=6). The increase in perfusion pressure was dependent on the degree of alveolar hypoxia. Figures 5B and 5C, respectively, show that the perfusion pressure increased from 7±1 mm Hg to 14±1 mm Hg when the alveoli were supplied with 2% O₂, and from 7±1 mm Hg to 11±1 mm Hg with 6% O₂ (n=4). The increase in perfusion pressure with 2% and 6% O₂, respectively, was abolished by preincubation (10 minutes) with 300 μmol/L 8-bromo-cADPR (Figures 5B and 5C; n=4).
Findings in the rat lung in situ were similar. Figure 8D shows the increase in perfusion pressure by 20 mmol/L K+ (from 7±1 to 10±1 mm Hg) and 75 mmol/L K+ (from 7±1 to 19±2 mm Hg). After preincubation (10 minutes) with 300 μmol/L 8-bromo-cADPR, 20 mmol/L K+ increased perfusion pressure from 7±1 to 10±1 mm Hg, and 75 mmol/L K+ increased perfusion pressure from 7±1 to 19±2 mm Hg (n=4). Figure 8B shows the increase in perfusion pressure by 1 μmol/L PGF2α (from 7±1 to 10±1 mm Hg) and by 3 μmol/L PGF2α (from 7±1 to 17±1 mm Hg). Preincubation (10 minutes) with 300 μmol/L 8-bromo-cADPR had no effect, because 1 and 3 μmol/L PGF2α increased perfusion pressure from 7±0.4 to 10±1 mm Hg and from 7±1 to 17±1 mm Hg, respectively (n=4).

Discussion

Previously, we showed that the level of enzyme activities for the synthesis and metabolism of cADPR in pulmonary artery smooth muscle is inversely related to artery diameter, as is the magnitude of the hypoxic constriction. We also showed that hypoxia increases cADPR accumulation in pulmonary artery smooth muscle, leading to SR Ca2+ release and constriction.

Figure 8. 8-Bromo-cADPR does not inhibit the constriction by K+ or to PGF2α in isolated pulmonary arteries or in the rat lung in situ. A, Constriction of an intact isolated pulmonary artery ring by 20 and 75 mmol/L K+, respectively, in the absence and presence of 1 μmol/L 8-bromo-cADPR. B, Response of an isolated pulmonary artery ring to hypoxia (16 to 21 Torr) in the absence and presence of 3 μmol/L 8-bromo-cADPR. C, Change in perfusion pressure in the rat lung in situ in response to alveolar hypoxia (2% O2) in the absence and presence of 3 μmol/L 8-bromo-cADPR. D, Change in perfusion pressure in the rat lung in situ in response to alveolar hypoxia (2% O2) in the absence and presence of 10 μmol/L 8-bromo-cADPR. B and D also show that the block of HPV by the minimum effective concentration of 8-bromo-cADPR is not reversed on washing.

The effect of 8-bromo-cADPR was selective for HPV over vasoconstriction by K+ and PGF2α, respectively. Figure 8A shows the constriction of an intact pulmonary artery ring by 20 mmol/L K+ (4±0.6 mN/mm) and by 75 mmol/L K+ (10±1 mN/mm). After preincubation (10 minutes) with 300 μmol/L 8-bromo-cADPR, constriction by 20 and 75 mmol/L K+ measured 4±0.3 and 10±1 mN/mm, respectively (n=4). Figure 8B shows constriction of an intact artery ring by 1 μmol/L PGF2α (3±0.5 mN/mm) and by 3 μmol/L PGF2α (8±1 mN/mm). After preincubation (10 minutes) with 300 μmol/L 8-bromo-cADPR, constriction by 1 and 3 μmol/L PGF2α remained unaffected, at 3±0.7 and 8±1 mN/mm, respectively (n=4).
To advance our proposal that cADPR promotes HPV, we further investigated the effect of 8-bromo-cADPR, a membrane-permeant cADPR antagonist, on acute HPV in isolated rat pulmonary arteries and in the perfused and ventilated rat lung in situ.

We first showed that phases 1 and 2 of HPV in isolated rat pulmonary arteries were abolished after depletion of ryanodine-sensitive SR Ca\(^{2+}\) stores with ryanodine and caffeine. In contrast, phase 1 of HPV remained unaffected in the presence of 8-bromo-cADPR. Thus, a cADPR-independent O\(_2\)-sensing mechanism must initiate ryanodine-sensitive SR Ca\(^{2+}\) release during phase 1 of HPV.\(^{9,15}\) Conversely, cyclopiazonic acid abolished phase 1 of HPV. This suggests that phase 1 of HPV in isolated arteries is mediated by the inhibition of SR Ca\(^{2+}\) ATPase activity, yielding net Ca\(^{2+}\) efflux from SR stores. When taken together with the fact that removing extracellular Ca\(^{2+}\) has no effect on the phase 1 constriction,\(^9\) this finding brings into question the proposal that phase 1 of HPV relies heavily on the activation, by hypoxia, of capacitative Ca\(^{2+}\) entry in the smooth muscle.\(^{19}\)

The maintained constriction associated with phase 2 of HPV in intact and de-endothelialized pulmonary artery rings was blocked by ryanodine, caffeine, and 8-bromo-cADPR. In contrast, it remained unaltered in the presence of the SR Ca\(^{2+}\) ATPase antagonist cyclopiazonic acid. Thus, HPV in isolated rat pulmonary arteries must be maintained by cADPR-dependent SR Ca\(^{2+}\) release alone and not by inhibition of SR Ca\(^{2+}\) ATPase.

When the endothelium was present, phase 2 of HPV in intact pulmonary artery rings was also abolished by 8-bromo-cADPR, but it was recovered when arteries were precontracted with K\(^+\) (ie, by voltage-gated Ca\(^{2+}\) influx). In marked contrast, block by 8-bromo-cADPR of the plateau constriction by hypoxia in pulmonary arteries without the endothelium was not reversed by K\(^+\)-induced preconstriction. Thus, we can conclude with some confidence that 8-bromo-cADPR blocked phase 2 of HPV in isolated arteries by inhibiting Ca\(^{2+}\) release from ryanodine-sensitive SR stores in the smooth muscle and that it did so without affecting (1) depolarization-induced Ca\(^{2+}\) influx or constriction by Ca\(^{2+}\) per se, (2) the release of the endothelium-derived vasoconstrictor, or (3) the increase in myofilament Ca\(^{2+}\) sensitivity promoted by the released vasoconstrictor.\(^8,20\) The release of physiological concentrations of the endothelium-derived vasoconstrictor during phase 2 of HPV\(^7,8,18,20\) is therefore unable to induce sufficient myofilament Ca\(^{2+}\) sensitization\(^8,20\) to promote constriction in isolated pulmonary arteries in the absence of maintained cADPR-dependent SR Ca\(^{2+}\) release.

These findings suggest that there are at least 3 discrete components to HPV in isolated rat pulmonary arteries: (1) inhibition of the smooth muscle SR Ca\(^{2+}\) ATPase, (2) activation by cADPR of SR Ca\(^{2+}\) release from ryanodine-sensitive SR stores, and (3) the release of endothelium-derived vasoconstrictor(s). Furthermore, we showed that 8-bromo-cADPR was without effect on components 1 and 3, but it abolished component 2. Therefore, we were able to investigate the relative importance of these key processes in triggering and maintaining HPV in the perfused and ventilated rat lung in situ. When added to the perfusate at concentrations ≥10 μmol/L, 8-bromo-cADPR blocked the initiation of acute HPV. Thus, cADPR-dependent SR Ca\(^{2+}\) release seems to be the primary trigger for acute HPV in the rat lung. Because phase 1 of HPV in isolated arteries remains unaffected in the presence of 8-bromo-cADPR, we can conclude that the mechanisms underpinning this phase of constriction (ie, inhibition of the SR Ca\(^{2+}\) ATPase) do not contribute greatly to HPV in the lung. It is surprising, therefore, that inhibition of the SR Ca\(^{2+}\) ATPase by hypoxia triggers such a pronounced constriction in isolated pulmonary arteries. One explanation for this could be that the change in O\(_2\) tension around the artery is faster in the myograph chamber than it is when associated with alveolar hypoxia in the lung. As a result, the former but not the latter may induce a transient fall in smooth muscle ATP levels,\(^{21}\) possibly due to delayed accommodation with respect to the energy state of the smooth muscle.

Our findings in isolated arteries also showed that 8-bromo-cADPR had no effect on the release or action of the endothelium-derived vasoconstrictor(s). Therefore, we can conclude that the release of physiological concentrations of the vasoconstrictor by hypoxia is unable to promote HPV in the lung in the absence of cADPR-dependent SR Ca\(^{2+}\) release in the smooth muscle of pulmonary arteries.

Previous studies have shown that nitric oxide production by the endothelium may first increase and then decline in response to hypoxia and that nitric oxide synthase inhibitors augment acute HPV.\(^{22-26}\) However, in the presence of 8-bromo-cADPR, hypoxia had no influence on resting tension in isolated arteries and had no effect on resting perfusion pressure in the rat lung. Nitric oxide may therefore act as a secondary modulator of HPV.

Our proposals are supported by the fact that, once triggered, HPV in the lung was completely reversed by concentrations of 8-bromo-cADPR that were unable to block phase 1 of HPV or the endothelium-dependent component of phase 2 of HPV in isolated arteries. Further support comes from the fact that the IC\(_{50}\) (55 μmol/L) for inhibition by 8-bromo-cADPR of HPV in the lung closely matched the IC\(_{50}\) (30 μmol/L) for inhibition by 8-bromo-cADPR of the ryanodine-sensitive plateau constriction by hypoxia in isolated arteries without the endothelium. The small difference between the measured IC\(_{50}\) in each preparation is likely due to differences in pharmacokinetics.

Surprisingly, 8-bromo-cADPR was able to block the initiation of HPV in isolated pulmonary arteries and in the rat lung at concentrations an order of magnitude lower than the concentrations required to completely reverse HPV after it had been initiated. This is all the more intriguing because, in contrast to its concentration-dependent reversal of HPV, preincubation with 8-bromo-cADPR blocked the initiation of HPV in an all-or-none manner but with a clear threshold concentration. This all-or-none block is reminiscent of the block by α-bungarotoxin of transmission at the neuromuscular junction. Thus, skeletal muscle fibers respond maximally to nerve stimulation when 45% of the nicotinic acetylcholine receptors are blocked. However, paralysis develops rapidly once any more than 45% of receptors are blocked.\(^{27}\) A similar “margin of safety” may be built into HPV, such that in pulmonary artery smooth muscle, a certain proportion of...
ryanodine receptors must be activated by cADPR to breach a given threshold for the initiation of a regenerative, global \( \text{Ca}^{2+} \) wave in the smooth muscle and, therefore, constriction. It would follow that activation by cADPR of fewer ryanodine receptors than is required to breach this threshold would be insufficient to trigger HPV. However, more detailed investigations will be required to confirm this proposal.

Finally, it is interesting to note that previous studies have found that the constriction by low concentrations of PGF\(_{2\alpha}\) can be inhibited by depletion of ryanodine-sensitive SR stores,\(^9\) although 8-bromo-cADPR had no effect on constriction by PGF\(_{2\alpha}\) or by \( \text{K}^+ \) in the present study. It seems likely, therefore, that PGF\(_{2\alpha}\) may, unlike hypoxia, access ryanodine-sensitive SR \( \text{Ca}^{2+} \) stores through a cADPR-independent mechanism (eg, calcium-induced calcium release by ryanodine receptors or IP\(_3\) induced SR \( \text{Ca}^{2+} \) release).

In conclusion, we propose that cADPR acts as the primary trigger for acute HPV in isolated rat pulmonary arteries and in the rat lung in situ. Our findings argue against a significant role for membrane depolarization and voltage-gated \( \text{Ca}^{2+} \) influx\(^3-6\) in acute HPV. The development of selective cADPR antagonists may therefore provide new and important therapeutic agents for the treatment of hypoxic pulmonary hypertension.

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**References**