Ca\(^{2+}\) release from ryanodine-sensitive store contributes to mechanism of hypoxic vasoconstriction in rat lungs

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Morio, Yoshiteru, and Ivan F. McMurtry. Ca\(^{2+}\) release from ryanodine-sensitive store contributes to mechanism of hypoxic vasoconstriction in rat lungs. J Appl Physiol 92: 527–534, 2002.—Studies of thapsigargin, cyclopiazonic acid, and ryanodine in isolated pulmonary arteries and smooth muscle cells suggest that release of Ca\(^{2+}\) from inositol 1,4,5-trisphosphate (IP\(_3\))- and/or ryanodine-sensitive sarcoplasmic reticulum Ca\(^{2+}\) stores is a component of the mechanism of acute hypoxic pulmonary vasoconstriction (HPV). However, the actions of these agents on HPV in perfused lungs have not been reported. Thus we tested effects of thapsigargin and cyclopiazonic acid, inhibitors of sarcoplasmic reticulum Ca\(^{2+}\)-ATPase, and of ryanodine, an agent that either locks the ryanodine receptor open or blocks it, on HPV in salt solution-perfused rat lungs. After inhibition of cyclooxygenase and nitric oxide synthase, thapsigargin (10 nM) and cyclopiazonic acid (5 μM) augmented the vasoconstriction to 0% but not to 3% inspired O\(_2\). Relatively high concentrations of ryanodine (100 and 300 μM) blunted HPV in nitric oxide synthase-inhibited lungs. The results indicate that release of Ca\(^{2+}\) from the ryanodine-sensitive, but not the IP\(_3\)-sensitive, store contributes to the mechanism of HPV in perfused rat lungs and that Ca\(^{2+}\)-ATPase-dependent Ca\(^{2+}\) buffering moderates the response to severe hypoxia.

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Ca\(^{2+}\) release from ryanodine-sensitive store contributes to mechanism of hypoxic vasoconstriction in rat lungs
ryanodine- and/or IP3-sensitive SR Ca\(^{2+}\) stores plays a role in the responses of isolated pulmonary arteries and PASMC to hypoxia. Further support for this idea is provided by recent reports that hypoxia increases PASMC levels of the endogenous ryanodine receptor agonist cADP-ribose (54) and that the cADP-ribose antagonist, 8-bromo-cADP-ribose, blocks the sustained hypoxic contractions of rabbit (54) and rat (8) pulmonary arteries and the hypoxic vasconstriction of perfused rat lungs (8). Thus it has been suggested that HPV is initiated and sustained by release of SR Ca\(^{2+}\) without a significant role for membrane depolarization and voltage-gated Ca\(^{2+}\) influx (8, 9, 33).

With the exception of the recent finding by Dipp and Evans (8) that 8-bromo-cADP-ribose inhibits hypoxic vasconstriction in rat lungs, the role of Ca\(^{2+}\) release from intracellular SR stores in the mechanism of HPV in perfused lungs has not been reported. Because much of the evidence for involvement of release of SR Ca\(^{2+}\) comes from studies of the effects of thapsigargin, cyclopiazonic acid, and ryanodine in PASMC and isolated pulmonary arteries, and because physiological and pharmacological disparity is often observed between isolated arteries and whole lungs (47), we believe it important to examine the effects of these agents on HPV in the more intact pulmonary vascular bed of the isolated, physiological salt solution (PSS)-perfused rat lung.

METHODS

Animals. Experiments were performed with adult male Sprague-Dawley rats (300–400 g) that were kept at Denver’s altitude of 5,280 ft (barometric pressure ~630 mmHg, inspired O\(_2\) tension ~122 Torr). The rats were housed under a 12:12-h light-dark cycle and allowed free access to standard rat food and water. All experimental procedures were approved by the Animal Care and Use Committee of the University of Colorado Health Sciences Center.

Isolated perfused lungs. Lungs were isolated from rats after anesthesia with intraperitoneal pentobarbital sodium (30 mg) and an intracardiac injection of 100 IU of heparin as previously described (11, 12, 25, 36, 37). Isolated lungs were ventilated with a humid mixture of 21% O\(_2\)-5% CO\(_2\)-74% N\(_2\) at 60 breaths/min, an inspiratory pressure of 9 cmH\(_2\)O, and an end-expiratory pressure of 2.5 cmH\(_2\)O. They were perfused through a main pulmonary artery cannula with perfluorocarbon (Earle’s balanced salt solution; Sigma Chemical). Ficoll (4 g/100 ml, type 70; Sigma Chemical) was included as a colloid, and 3.1 mM sodium meclofenamate (Sigma Chemical) was added to inhibit synthesis of vasodilator prostaglandins (27, 36). After lungs were flushed of blood with 20 ml of PSS, they were perfused with a recirculated volume of 30 ml. Effluent perfusate drained from a left ventricular cannula into a perfusate reservoir located below the level of the lung. The perfusate reservoir volume was continuously monitored, and any lung preparation that leaked perfusate or became overtly edematous was excluded from the study. Lung and perfusate temperatures were maintained at 37°C, and perfusate pH was kept between 7.3 and 7.4.

The PSS-perfused lungs were equilibrated for 20 min before two hypoxic pressor responses were elicited by ventilation with either 0% O\(_2\)-5% CO\(_2\)-95% N\(_2\) or 3% O\(_2\)-5% CO\(_2\)-92% N\(_2\) for 10 min at 20-min intervals to induce hypoxic vasoreactivity. Subsequent hypoxic pressor responses were elicited by ventilation with either the 0% or 3% O\(_2\)-gas mixtures before and after treatment with either vehicles (controls) or inhibitors of SR Ca\(^{2+}\) release. Previous in-line O\(_2\) electrode measurements have shown effluent perfusate O\(_2\) tensions of ~118, 20, and 3 Torr, respectively, during ventilation of this preparation with 21, 3, and 0% O\(_2\) (37). Because of some oxygenation of the recirculated perfusate by room air in the unsealed reservoir and possibly through the walls of the tubing, the corresponding O\(_2\) tensions of the influent perfusate were ~120, 46, and 33 Torr. Because the lungs were ventilated at constant airway pressures and perfused at constant flow under zone 2 conditions, changes in perfusion pressure reflected changes in pulmonary vascular resistance. The vascular effects of the inhibitors of SR Ca\(^{2+}\) release were analyzed by measuring baseline (normoxic) perfusion pressure and the peak of the hypoxic pressor response. In addition, the percent spontaneous reversal (dilation) of the hypoxic vasoconstriction that occurred within 2 min of the peak of the response (11, 12) was measured during the second hypoxic response elicited 40 min after treatment with either vehicle or inhibitor. To control for differences in vascular reactivity over time of perfusion and with repeated hypoxic challenges, SR Ca\(^{2+}\) inhibitor lungs and the respective vehicle control lungs were treated identically with respective to time and hypoxic challenges.

Experimental protocols. Because thapsigargin and cyclopiazonic acid stimulate endothelial NO synthesis (24, 25, 56), the first set of experiments compared the separate effects of the two inhibitors of SR Ca\(^{2+}\)-ATPase on HPV in PSS-perfused rat lungs either pretreated or not with the inhibitor of NO synthase nitro-L-arginine (l-NAME) (Aldrich). After equilibration and two initial challenges with 0% O\(_2\), lungs were treated by adding either 60 μl saline (vehicle control) or 200 μM l-NAME (25, 36) to the perfusate. Fifteen minutes later, a third hypoxic challenge was given, and then either DMSO (vehicle; 30 μl), thapsigargin (Calbiochem; 10 nM) (4, 25), or cyclopiazonic acid (Calbiochem; 5 μM) (8, 10, 13, 16) was added to the perfusate. After an additional 15 min, the lungs were again challenged twice with 0% O\(_2\) ventilation for 15 min, with 10 min of normoxic ventilation between the two challenges.

Although there is no clear evidence that ryanodine stimulates NO synthesis, the second experiment compared effects of the ryanodine-receptor inhibitor on the response to 0% O\(_2\) in lungs either pretreated or not with l-NAME to control for any possible interaction between NO and ryanodine-sensitive Ca\(^{2+}\) stores (28, 35). The protocol was identical to that described above for the Ca\(^{2+}\)-ATPase inhibitors except either 30 μl distilled H\(_2\)O (vehicle) or 10 μM ryanodine (10, 13, 15, 18, 33, 45) was added to the perfusate of untreated and l-NAME-pretreated lungs 15 min before the final two hypoxic challenges. The effects of a fivefold higher concentration of ryanodine (50 μM) (30, 35) were also examined in additional l-NAME-pretreated lungs.

To test whether a possible role of release of intracellular Ca\(^{2+}\) in HPV might depend on the severity of hypoxia, a third experiment examined the separate effects of thapsigargin and ryanodine in l-NAME-pretreated lungs challenged with 3% O\(_2\) instead of 0% O\(_2\). The protocol was as described in the preceding paragraphs except lungs were challenged with 3%
Results

In the first experiment, cyclopiazonic acid, but not thapsigargin, caused a slight increase in baseline (normoxic) perfusion pressure in lungs either pretreated or not with L-NNA (Table 1). Whereas both agents reduced the peak of the pressor response to 0% O₂ in lungs not pretreated with L-NNA, they augmented the response in lungs pretreated with the NO synthase inhibitor (Fig. 1). Representative perfusion pressure tracings in L-NNA-pretreated lungs are shown in Fig. 2. Although both thapsigargin and cyclopiazonic acid augmented the peak of the hypoxic response, neither agent prevented the marked spontaneous reversal (dilation) of the vasoconstriction (Fig. 2 and Table 2).

![Graph showing perfusion pressure changes](image_url)

Fig. 1. Both thapsigargin (TG, 10 nM) and cyclopiazonic acid (CPA, 5 μM) reduced the hypoxic pressor responses (HPR) to 0% inspired O₂ in lungs not treated with the NO synthase inhibitor nitro-L-arginine (L-NNA) but augmented the larger HPR in lungs pretreated with 200 μM L-NNA (+L-NNA). DM1SO (30 μl) was added to perfusate of vehicle control lungs. Time after treatment is minutes after addition of either vehicle, TG, or CPA to lung perfusate. Values are means ± SE; nos. in parentheses, no. of lungs. *P < 0.05 for both TG and CPA groups vs. vehicle control group by ANOVA. †P < 0.05 for TG group vs. vehicle control group by ANOVA.

Table 1. Effects of TG, CPA, and RD on baseline perfusion pressure during normoxic (21% O₂) ventilation in lungs either pretreated or not with the NO synthase inhibitor nitro-L-arginine

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>n</th>
<th>0</th>
<th>15</th>
<th>40</th>
<th>Δ Pressure, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>-L-NNA</td>
<td>DMSO</td>
<td>4</td>
<td>7.5 ± 0.3</td>
<td>8.0 ± 0.4</td>
<td>8.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TG</td>
<td>4</td>
<td>6.4 ± 0.5</td>
<td>7.0 ± 0.6</td>
<td>7.7 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CPA</td>
<td>4</td>
<td>6.9 ± 0.4</td>
<td>8.2 ± 0.3</td>
<td>9.1 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>+L-NNA</td>
<td>DMSO</td>
<td>7</td>
<td>6.3 ± 0.4</td>
<td>7.1 ± 0.4</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TG</td>
<td>8</td>
<td>6.0 ± 0.4</td>
<td>7.6 ± 0.5</td>
<td>8.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CPA</td>
<td>5</td>
<td>6.2 ± 0.3</td>
<td>8.8 ± 0.7</td>
<td>9.9 ± 0.6#</td>
</tr>
<tr>
<td>Second</td>
<td>-L-NNA</td>
<td>H₂O</td>
<td>4</td>
<td>6.1 ± 0.4</td>
<td>6.8 ± 0.4</td>
<td>7.4 ± 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RD (10 μM)</td>
<td>4</td>
<td>6.1 ± 0.3</td>
<td>6.7 ± 0.4</td>
<td>7.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>+L-NNA</td>
<td>H₂O</td>
<td>5</td>
<td>6.4 ± 0.5</td>
<td>7.1 ± 0.5</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RD (10 μM)</td>
<td>6</td>
<td>6.8 ± 0.3</td>
<td>7.7 ± 0.3</td>
<td>8.7 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H₂O</td>
<td>5</td>
<td>7.0 ± 0.3</td>
<td>7.9 ± 0.3</td>
<td>9.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RD (50 μM)</td>
<td>5</td>
<td>7.7 ± 0.2</td>
<td>9.1 ± 0.4</td>
<td>10.1 ± 0.5</td>
</tr>
<tr>
<td>Third</td>
<td>+L-NNA</td>
<td>DMSO</td>
<td>7</td>
<td>7.3 ± 0.3</td>
<td>8.1 ± 0.3</td>
<td>9.2 ± 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TG</td>
<td>7</td>
<td>7.8 ± 0.8</td>
<td>9.2 ± 1.0</td>
<td>9.7 ± 0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H₂O</td>
<td>5</td>
<td>6.5 ± 0.5</td>
<td>7.2 ± 0.2</td>
<td>7.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RD (10 μM)</td>
<td>5</td>
<td>6.6 ± 0.1</td>
<td>7.2 ± 0.1</td>
<td>7.9 ± 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H₂O</td>
<td>5</td>
<td>7.0 ± 0.4</td>
<td>9.1 ± 0.9</td>
<td>9.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RD (100 μM)</td>
<td>5</td>
<td>7.3 ± 0.3</td>
<td>9.5 ± 0.8</td>
<td>10.1 ± 0.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>H₂O</td>
<td>3</td>
<td>7.4 ± 0.2</td>
<td>8.7 ± 0.4</td>
<td>9.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>RD (300 μM)</td>
<td>3</td>
<td>7.2 ± 0.2</td>
<td>8.9 ± 0.3</td>
<td>9.4 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of lungs. L-NNA, nitro-L-arginine; DMSO, dimethyl sulfoxide (vehicle); TG, thapsigargin (10 nM); CPA, cyclopiazonic acid (5 μM); H₂O, distilled water (vehicle); RD, ryanodine (10, 50, 100, and 300 μM). *P < 0.05 for CPA value vs. DMSO (vehicle control) value by ANOVA.
In the second experiment, neither 10 nor 50 μM ryanodine had any effect on either baseline perfusion pressure (Table 1) or the pressor response to 0% O₂ in lungs either pretreated or not with L-NNA (Fig. 3). Ryanodine also did not affect the spontaneous reversal of hypoxic vasoconstriction in L-NNA-pretreated lungs (Fig. 2 and Table 2).

Table 2. Effects of TG, CPA, and RD on spontaneous reversal (dilation) of hypoxic vasoconstriction in nitro-L-arginine-pretreated lungs

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Treatment</th>
<th>n</th>
<th>Spontaneous Reversal, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>DMSO</td>
<td>7</td>
<td>44.0 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>8</td>
<td>38.4 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>CPA</td>
<td>5</td>
<td>33.6 ± 6.5</td>
</tr>
<tr>
<td></td>
<td>H₂O</td>
<td>5</td>
<td>39.0 ± 8.2</td>
</tr>
<tr>
<td></td>
<td>RD (10 μM)</td>
<td>6</td>
<td>52.2 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>H₂O</td>
<td>5</td>
<td>32.8 ± 3.0</td>
</tr>
<tr>
<td></td>
<td>RD (50 μM)</td>
<td>5</td>
<td>29.0 ± 4.6</td>
</tr>
<tr>
<td>Third</td>
<td>DMSO</td>
<td>7</td>
<td>11.3 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>7</td>
<td>9.1 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>H₂O</td>
<td>5</td>
<td>11.4 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>RD (10 μM)</td>
<td>5</td>
<td>8.0 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>H₂O</td>
<td>5</td>
<td>18.7 ± 5.2</td>
</tr>
<tr>
<td></td>
<td>RD (100 μM)</td>
<td>5</td>
<td>20.4 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>H₂O</td>
<td>3</td>
<td>8.7 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>RD (300 μM)</td>
<td>3</td>
<td>30.8 ± 2.0*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of lungs. DMSO, vehicle; TG, 10 nM; CPA, 5 μM; H₂O, vehicle; RD, 10, 50, 100, and 300 μM. Spontaneous reversal of second hypoxic pressor response elicited 40 min after treatment with either vehicle or inhibitor was calculated as percent decrease in pressor response within 2 min of the peak of the response to either 0% O₂ (first and second experiments) or 3% O₂ (third experiment). *P < 0.05 for RD (300 μM) value vs. respective H₂O (vehicle control) value by unpaired t-test.

In the third experiment with L-NNA-pretreated lungs challenged with 3% O₂ instead of 0% O₂, 10 nM thapsigargin neither increased baseline perfusion pressure (Table 1) nor affected either the peak (Fig. 4) or the spontaneous reversal (Fig. 2 and Table 2) of the hypoxic pressor response. Whereas neither 10, 100, nor 300 μM ryanodine affected baseline perfusion pressure (Table 1), 100 μM ryanodine tended to blunt the peak hypoxic vasoconstriction, and the inhibition by 300 μM was statistically significant (Fig. 5). The percent spontaneous reversal of hypoxic vasoconstriction was increased in lungs treated with 300 μM but not with 100 μM, ryanodine (Table 2). Representative perfusion pressure tracings of effects of 300 μM ryanodine on HPV are shown in Fig. 2. In lungs treated with 100 μM ryanodine, 0.1 μM nifedipine caused immediate and marked inhibition of the residual, ongoing hypoxic vasoconstriction (Fig. 6).

DISCUSSION

This study showed that the effects of thapsigargin and cyclopiazonic acid on HPV in PSS-perfused rat lungs depended on whether lungs had been pretreated with an inhibitor of NO synthase and on the severity of hypoxic challenge. Whereas both inhibitors of SR Ca²⁺-ATPase blunted the pressor response to 0% O₂ in the absence of L-NNA, they augmented the response after inhibition of NO synthesis. However, thapsigargin did not augment the pressor response to 3% O₂ in L-NNA-pretreated lungs. Because blunting of HPV by thapsigargin and cyclopiazonic acid in the absence of L-NNA was apparently due to increased synthesis of the vasodilator NO (24, 25, 56), these results provide no evidence that release of Ca²⁺ from IP₃-sensitive SR stores

Fig. 2. Computer scans of representative recordings of HPV before and 15 and 40 min after addition of either vehicle (VEH, DMSO for TG and CPA and distilled H₂O for ryanodine), 10 nM TG, 5 μM CPA, or 50 and 300 μM ryanodine (RD) to perfusate of lungs pretreated with 200 μM L-NNA. TG and CPA augmented peak pressor response to 0% inspired O₂ (A). Neither agent prevented the spontaneous reversal (dilation) of the vasoconstriction to 0% O₂. In contrast to the effect on response to 0% O₂, TG did not augment response to 3% O₂ (C). Whereas 50 μM ryanodine did not affect pressor response to 0% O₂ (B), 300 μM ryanodine blunted response to 3% O₂ (D). Bars at bottom show duration of hypoxic challenges that were 10 min before and 15 min at 15 and 40 min after treatment.
is an important component of the mechanism of HPV in perfused rat lungs. In contrast, relatively high concentrations of ryanodine blunted HPV in L-NNA-pretreated lungs, a finding consistent with the possibility that release of Ca\(^{2+}\) from the ryanodine-sensitive, CICR SR store contributes to the overall mechanism of HPV.

A limitation of this study is that we had no independent measure of the effects of thapsigargin and cyclopiazonic acid or of ryanodine on release of SR Ca\(^{2+}\) in the PASMC of the perfused lung. The 5 \(\mu\)M concentration of cyclopiazonic acid used in our study was similar to that used to inhibit Ca\(^{2+}\)-ATPase and deplete IP\(_{3}\)-sensitive Ca\(^{2+}\) stores in isolated arteries and smooth muscle cells (8, 10, 13, 16). Because concentrations of thapsigargin >30 nM caused edema in PSS-perfused rat lungs (4), we limited its concentration to 10 nM, which was lower than the 1–5 \(\mu\)M generally used in vitro (10, 13, 16). However, our laboratory has previously observed that 10 nM thapsigargin causes contraction of isolated rat pulmonary arteries (25), which suggests that this concentration is sufficient to inhibit Ca\(^{2+}\)-ATPase in rat conduit PASMC. Despite the different concentrations, the two inhibitors of Ca\(^{2+}\)-ATPase had very similar effects on pulmonary vasoreactivity. Only cyclopiazonic acid caused a slight increase in baseline perfusion pressure, but both agents blunted the pressor response to 0% \(O_2\) in the absence of L-NNA and augmented the response in the presence of L-NNA. Although the 10 \(\mu\)M concentration of ryanodine used in our initial experiments has been commonly used in studies of isolated arteries and PASMC (6, 10, 13, 15, 18, 33, 45) and has been found in some cases to inhibit hypoxic responses (6, 10, 13, 18, 45), it had no effect on HPV in the perfused lungs. Because some reports indicate that concentrations as high as 300 \(\mu\)M are required to inhibit release of Ca\(^{2+}\) from the CICR store (14), we also examined the effects of 100 and 300 \(\mu\)M ryanodine and observed significant blunting of HPV by the highest concentration. We attempted in preliminary experiments to use caffeine-induced vasoconstriction to test for the effectiveness of ryanodine blockade of CICR (13, 14, 33) but found that 10 mM caffeine caused vasodilation rather than vasoconstriction in meclofenamate- and L-NNA-pretreated rat lungs (unpublished observations).

Our observation that thapsigargin and cyclopiazonic acid augmented the pressor response to 0% \(O_2\) is similar to that of Jabr et al. (13), who observed potentiation of hypoxic (0% \(O_2\)) contraction of dog pulmonary arteries by these inhibitors of SR Ca\(^{2+}\)-ATPase. A difference between the studies is that we found potentiation of the hypoxic response only after inhibition of NO synthesis, whereas a NO synthase inhibitor was not used in the isolated pulmonary arteries. The arteries were isolated with functional endothelium (13), and it is unclear why endothelial NO production in response to the Ca\(^{2+}\)-ATPase inhibitors (24, 25, 56) was apparently not an issue. Because the larger hypoxic contractions of isolated arteries after thapsigargin or cyclopiazonic acid were less sensitive to inhibition by L-type Ca\(^{2+}\) channel blockers than were the control responses, Jabr et al. (13) speculated that additional Ca\(^{2+}\) influx through a dihydropyridine-insensitive pathway contributed to the potentiation. We did not examine this possibility in the perfused lungs, and the potentiation of HPV might have been due to a similar mechanism and/or to a reduction in the ability of SR Ca\(^{2+}\)-ATPase to buffer an increase in cytosolic Ca\(^{2+}\) (46). We do not know why we and Jabr et al. observed potentiation of hypoxic responses, whereas Dipp and Evans (8), Gelband and Gelband (10), and Robertson et al. (33) found inhibition of hypoxic contraction of iso-

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**Fig. 4.** TG (10 nM) had no effect on HPR to 3% inspired \(O_2\) in lungs pretreated with 200 \(\mu\)M L-NNA. Time after treatment is minutes after addition of either vehicle (DMSO) or TG to perfusate. Values are means ± SE; nos. in parentheses, no. of lungs.

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lated rat pulmonary arteries by thapsigargin and/or cyclopiazonic acid.

We are also uncertain why thapsigargin potentiated HPV to 0% O2 but not to 3% O2 in l-NNNA-pretreated lungs. It can be speculated that if potentiation of the peak response to 0% O2 by thapsigargin were due to inhibition of Ca2+-buffering by SR Ca2+-ATPase (46), then possibly the buffering was greater during the response to 0% O2 than during that to 3% O2. In view of evidence that severe hypoxia stimulates Ca2+-ATPase-dependent Ca2+ uptake in rabbit pulmonary arteries (49), such a difference is feasible. In contrast, potentiation of the peak response to 0% O2 by the inhibitors of Ca2+-ATPase was apparently not due to attenuation of the spontaneous reversal (dilation) of the response, because the dilation was not reduced by either thapsigargin or cyclopiazonic acid. This agrees with evidence that the spontaneous reversal of vasoconstriction to severe hypoxia in perfused lungs is due largely to stimulation of ATP-sensitive and Ca2+-activated K+ channels and the resulting membrane hyperpolarization and inhibition of voltage-gated Ca2+ influx (11, 12, 53).

Previous studies of effects of ryanodine on hypoxic contraction of isolated arteries have produced mixed results. Gelband and Gelband (10) and Liu et al. (18) found complete blockade of hypoxic contraction of rat and pig pulmonary arteries by 5 and 10 μM ryanodine, respectively, and Jabr et al. (13) observed blunting of hypoxic contraction of dog pulmonary arteries. In contrast, Jin et al. (15) reported no effect of 10 μM ryanodine on hypoxic contraction of rat pulmonary arteries. Saqueton et al. (35) infused ryanodine into the pulmonary artery of fetal lambs and observed that although it inhibited inhaled NO-induced pulmonary vasodilation, it had no effect on hypoxic pulmonary vascular tone. Whereas Dipp and Evans (8) reported complete blockade of hypoxic contraction of rat pulmonary arteries by 10 μM ryanodine plus 10 mM caffeine, this combination caused only slight inhibition in the study of Robertson et al. (33). There is no evident explanation for these disparate findings. In our study of perfused rat lungs, 10 μM ryanodine had no effect on HPV, 100 μM tended to reduce the response, and 300 μM caused significant blunting.

Our finding of blunting instead of blockade of HPV by a high concentration of ryanodine differs somewhat from the recent observation that HPV in rat lungs is both completely prevented and reversed by the cADP-ribose antagonist 8-bromo-cADP-ribose (8). Because hypoxia increases cADP-ribose in pulmonary artery smooth muscle (54), cADP-ribose induces Ca2+ release from ryanodine-sensitive stores (54), and 8-bromo-cADP-ribose blocks sustained hypoxic contraction of isolated pulmonary arteries and hypoxic vasoconstriction of perfused lungs (8, 54), Dipp and Evans (8) have proposed that cADP-ribose-induced release of SR Ca2+ initiates and sustains HPV without a significant role for membrane depolarization and voltage-gated Ca2+

![Diagram](image-url)
influx. Although these investigators present a strong case that 8-bromo-cADP-ribose-induced inhibition of HPV is due to inhibition of release of SR Ca$^{2+}$, our findings that high concentrations of ryanodine failed to abolish the hypoxic response and that the residual hypoxic vasoconstriction was immediately and markedly reversed by the voltage-gated Ca$^{2+}$ channel blocker nifedipine raise a note of caution. It would seem either that ryanodine failed to completely block CICR in our study or that 8-bromo-cADP-ribose had effects other than or in addition to inhibition of Ca$^{2+}$ release in the study of Dipp and Evans. With respect to the latter, one possibility that could be considered is that 8-bromo-cADP-ribose reversed a Ca$^{2+}$-ribosemediated inhibition of a hyperpolarizing K$^+$ current. For example, cADP-ribose has been reported to inhibit Ca$^{2+}$-activated K$^+$ channels in coronary arterial smooth muscle (17) and the activation of a voltage-sensitive K$^+$ current in neuroblastoma/glioma hybrid cells (3). Thus we suggest that further work is necessary to test whether 8-bromo-cADP-ribose-induced blockade of HPV in perfused rat lungs is due partly to activation of a hyperpolarizing K$^+$ current and inhibition of Ca$^{2+}$ influx rather than solely to inhibition of release of SR Ca$^{2+}$. Such a mechanism would be similar to the inhibition of HPV by blockade of the endothelin-1 ETA receptor that appears to be mediated through derepression of the ATP-sensitive K$^+$ channel (36).

If, as our results suggest, both CICR and voltage-gated Ca$^{2+}$ influx contribute to the intracellular Ca$^{2+}$ signal mediating HPV in PSS-perfused rat lungs, then the question arises as to which comes first. Some studies of PASMC indicate that hypoxia-induced release of intracellular Ca$^{2+}$ is the initiating event (10, 29, 34, 45). However, voltage-gated Ca$^{2+}$ influx is a physiological trigger for ryanodine receptor-mediated CICR in vascular smooth muscle (7), and Cornfield et al. (6) have proposed that hypoxia-induced membrane depolarization and Ca$^{2+}$ influx trigger CICR in fetal PASMC.

In summary, our results in PSS-perfused rat lungs do not support evidence from studies of effects of thapsigargin and cyclopiazonic acid in PASMC and isolated pulmonary arteries (8, 10, 33, 34) that Ca$^{2+}$ release from an IP$_3$-sensitive SR Ca$^{2+}$ store is an important component of the mechanism of HPV. However, our finding that relatively high concentrations of ryanodine blunt HPV agrees in principle with some studies of PASMC and isolated arteries (6, 8–10, 13, 18, 29, 45) and is consistent with the idea that Ca$^{2+}$ release from a ryanodine-sensitive SR Ca$^{2+}$ store contributes to the hypoxia-induced Ca$^{2+}$ signaling and vasoconstriction. What remains unclear are the relative roles and temporal relationships of voltage-gated Ca$^{2+}$ influx, CICR, voltage-independent Ca$^{2+}$ entry (33, 50), and increased Ca$^{2+}$ sensitivity of contractile myofilaments (20, 32) in the overall mechanism of HPV, and to what extent the contribution of these various components is species and/or experimental preparation dependent. As emphasized in recent editorials by Sylvester (42) and Weissmann et al. (52), many questions concerning the exact sequence of inter- and intracellular events underlying the mechanism of HPV remain to be definitively answered.

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REFERENCES


