EDHF-mediated vasodilation involves different mechanisms in normotensive and hypertensive rat lungs

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Morio, Yoshiteru, Ethan P. Carter, Masahiko Oka, and Ivan F. McMurtry. EDHF-mediated vasodilation involves different mechanisms in normotensive and hypertensive rat lungs. Am J Physiol Heart Circ Physiol 284: H1762–H1770, 2003. First published January 9, 2003; 10.1152/ajpheart.00831.2002.—The role of endothelium-derived hyperpolarizing factor (EDHF) in regulating the pulmonary circulation and the participation of cytochrome P-450 (CYP450) activity and gap junction intercellular communication in EDHF-mediated pulmonary vasodilation are unclear. We tested whether tonic EDHF activity regulated pulmonary vascular tone and examined the mechanism of EDHF-mediated pulmonary vasodilation induced by thapsigargin in salt solution-perfused normotensive and hypoxia-induced hypertensive rat lungs. After blockade of both cyclooxygenase and nitric oxide synthase, inhibition of EDHF with charybdotoxin and apamin, and it is now generally believed that the blockade is due to inhibition of endothelial cell hyperpolarization and generation of the EDHF signal (12, 17, 19). Whereas there is evidence that epoxygenesatrienoic acids (EETs) (5, 26, 44), potassium ions (3, 19), and hydrogen peroxide (36) can act as an EDHF in some arteries, the identity of EDHF remains controversial and may differ among species and vascular segments (6, 15, 20, 22, 55).

Although EETs may not be EDHF, several studies suggest that cytochrome P-450 (CYP450) activity is involved in the mechanism of EDHF-mediated vasodilation (6, 22, 43, 45). For example, sulfaphenazole, an inhibitor of cytochrome 2C isozymes, inhibits EDHF-mediated vasorelaxation in the porcine coronary artery (21) and 7-ethoxyresorufin, an inhibitor of CYP450 1A isozyme, inhibits the vasodilation in perfused rat mesenteric (1) and renal (2) vascular beds and the guinea pig cerebral artery (14). However, other studies in various arteries show that these and other CYP450 inhibitors fail to inhibit EDHF-mediated vasorelaxation, or do so nonspecifically, and do not support the involvement of CYP450 in the EDHF mechanism (18, 23, 24, 32, 36, 42, 58).

Another body of work suggests that gap junction intercellular communication plays a role in EDHF-mediated vasodilation (6, 15, 20, 55). Gap junctions are clusters of intercellular channels between adjacent cells that allow passage of ions and small molecules between the cells without involvement of extracellular space. The channels are formed by the docking of two connexons, each comprising six connexin protein subunits. Different connexin subtypes (e.g., Cx37, Cx40, and Cx43) are conventionally found in vascular endothelial and smooth muscle cells according to vessel type and species (57). Gap junctions connect adjacent endothelial cells, adjacent smooth muscle cells, and endothelial cells to smooth muscle cells. The incidence of myoendothelial gap junctions is higher in resistance than in conduit arteries (46). Putative gap junction inhibitors, such as palmitoleic acid (13, 27, 56), 18-glycyrrhetinic acid (10, 16, 23, 29, 36, 52, 53), and Gap 27 (9, 16, 47, 52), a synthetic peptide with sequence homology to a region of the second extracellular loop of Cx37 and Cx43, reduce EDHF-mediated hyperpolarization and relaxation in various arteries. These observations suggest that the EDHF signal represents electrotonic spread of hyperpolarization from the endo-

NUMEROUS STUDIES in various systemic arteries have investigated the nitric oxide (NO)- and prostacyclin (PGI2)-independent vasodilation mediated by endothelium-derived hyperpolarizing factor(s) (EDHF) (6, 15, 20, 22, 55). EDHF hyperpolarizes the smooth muscle, inhibits voltage-gated Ca2+ influx, and elicits vasodilation. EDHF-mediated vasodilation is blocked by the combined treatment with the Ca2+-activated K+ channel blockers charybdotoxin and apamin, and it is now generally believed that the blockade is due to inhibition of endothelial cell hyperpolarization and generation of the EDHF signal (12, 17, 19). Whereas there is evidence that epoxygenesatrienoic acids (EETs) (5, 26, 44), potassium ions (3, 19), and hydrogen peroxide (36) can act as an EDHF in some arteries, the identity of EDHF remains controversial and may differ among species and vascular segments (6, 15, 20, 22, 55).

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thelium into the medial smooth muscle via myendothelial and homocellular smooth muscle gap junctions.

Studies in isolated pulmonary arteries (11, 25, 31, 34, 40, 54) and perfused lungs (7, 28) report EDHF activity, and there is evidence for involvement of a CYP450 metabolite (31, 34). However, the physiological role of EDHF in the pulmonary circulation and the roles of CYP450 and gap junctions in EDHF-mediated pulmonary vasodilation are unclear. Thus the objectives of this study were to investigate whether EDHF activity contributed to the regulation of either basal or hypoxic pulmonary vascular tone and to examine the mechanism of EDHF-mediated pulmonary vasodilation in isolated normotensive and hypoxia-induced hypertensive rat lungs. After inhibition of both PGI2 and NO synthesis, we tested the effects of inhibitors of EDHF (charybdotoxin plus apamin), CYP450 (7-ethoxyresorufin and sulfaphenazole), and gap junctions (18-glycyrrehetic acid and palmitoleic acid) on basal perfusion pressure and acute hypoxic vasoconstriction and on the vasodilation induced by thapsigargin during hypoxic vasoconstriction.

METHODS

Animals. Experiments were performed with two groups of adult male Sprague-Dawley rats (250–350 g). The pulmonary normotensive group was kept at the Denver, CO, altitude of 5,280 ft (barometric pressure ~630 mmHg; inspired O2 tension ~122 mmHg). The chronically hypoxic, pulmonary hypertensive group was exposed to hypobaric hypoxia (barometric pressure ~410 mmHg; inspired O2 tension ~76 mmHg) for 3–4 wk in a chamber flushed continuously with room air to prevent accumulation of CO2, NH3, and H2O. Hypobaric exposure was 24 h/day except when the chamber was opened briefly to remove the rats or clean cages and replenish the food and water. All rats were housed under a 12:12-h light-dark cycle and allowed free access to standard rat food and water. The experimental procedures were approved by the Animal Care and Use Committee of the University of Colorado Health Sciences Center.

Isolated perfused lungs. After removal from the hypobaric chamber, pulmonary hypertensive rats were kept hypoxic in a small plastic box flushed with 10% O2 or, after anesthesia with intraperitoneal pentobarbital sodium (30 mg), by ventilation with 10% O2 until lungs were isolated. Lungs were isolated from the anesthetized rats after intracardiac injection of 100 IU heparin. The techniques of lung isolation, ventilation, and perfusion have been described in detail (37). The physiological salt solution (PSS) perfusate contained (in mM) 116.3 NaCl, 5.4 KCl, 0.83 MgSO4, 19.0 NaHCO3, 1.04 NaH2PO4, 1.8 CaCl2-2H2O, and 5.5 D-glucose (Earle’s balanced salt solution; Sigma). Ficoll (4 g/100 ml, type 70; Sigma) was included as a colloid, and 3.1 μM sodium meclofenamate (Sigma) was added to inhibit PGI2 synthesis (28, 41).

PSS-perfused normotensive and hypoxic hypertensive lungs were equilibrated at 37°C for 20 min during ventilation with 21% O2-5% CO2-74% N2 and 8% O2-5% CO2-87% N2, respectively. After equilibration, two hypoxic pressor responses were elicited by 10 min of ventilation with 0% O2-5% CO2-95% N2 and 3% O2-5% CO2-92% N2, separated by 10 min of normoxic ventilation, to induce hypoxic vasoactivity. Subsequent hypoxic pressor responses were elicited by ventilation with the 3% O2 gas mixture before and after treatment with either inhibitors or the respective vehicles (vehicle-time controls). Vascular effects of inhibitors were analyzed by measuring baseline perfusion pressure, peak hypoxic pressor response, and percent spontaneous (vehicle) or thapsigargin-induced vasodilation of the hypoxic vasoconstriction. Percent vasodilation was calculated by dividing the decrease in pressure that occurred 2 min after the addition of either vehicle or thapsigargin to the perfusate at the peak of the hypoxic response by the magnitude of the hypoxic response (28). To control for differences in vasoreactivity over time of perfusion and with repeated hypoxic challenges, inhibitor lungs and the respective vehicle time-control lungs were treated identically with respect to time and hypoxic challenges.

Tonic and stimulated EDHF activity. To eliminate involvement of both PGI2 and NO in the regulation of baseline vascular tone and acute hypoxic vasoconstriction, all normotensive and hypertensive lungs, which were exposed to the cyclooxygenase inhibitor meclofenamate at the beginning of perfusion, were treated with the NO synthase (NOS) inhibitor Nω-nitro-l-arginine (l-NNA, 200 μM) (Aldrich) after the first two hypoxic challenges (38, 41). As previously reported, this acute inhibition of NOS causes vasoconstriction in hypertensive but not normotensive lungs and augments subsequent hypoxic vasoconstriction in both groups of lungs (38, 41). The vasoconstriction in hypertensive lungs is reflected in the higher “baseline” perfusion pressures shown in Tables 1 and 2. Fifteen minutes after the addition of l-NNA, lungs were challenged twice with 3% O2 for 10 min and separated by 10 min of normoxic ventilation. To test for tonic EDHF-mediated regulation of “baseline” vascular tone and acute hypoxic vasoconstriction after inhibition of both PGI2 and NO synthesis, these lungs were then treated with the combination of 50 nM charybdotoxin (Sigma) plus 50 nM apamin (Sigma) (7, 16) or 30 μl saline (vehicle control) and challenged with 10 min of 3% O2 20 min later. The effect of stimulated EDHF activity was then examined by the addition of either 50 nM thapsigargin (Calbiochem) (38) or 15 μl DMSO (vehicle control) to the perfusate of the charybdotoxin plus apamin- and saline (vehicle)-treated lungs at the peak of the hypoxic pressor response.

Role of CYP450 activity. To test the effects of CYP450 inhibitors on “baseline” vascular tone and acute hypoxic vasoconstriction in meclofenamate- and l-NNA-pretreated normotensive lungs, 3 μM 7-ethoxyresorufin (Sigma) (1), 10 μM sulfaphenazole (Sigma) (21), or 30 μl DMSO (vehicle control) was added to the perfusate 5 min after the second post-l-NNA hypoxic response, and lungs were then challenged twice.

Table 1. The combination of CTX and apamin has no effect on baseline perfusion pressure in meclofenamate- and Nω-nitro-l-arginine-pretreated normotensive and hypoxic hypertensive lungs

<table>
<thead>
<tr>
<th></th>
<th>Baseline Perfusion Pressure, mmHg</th>
<th>n</th>
<th>0 min</th>
<th>20 min after treatment</th>
<th>ΔPressure, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive lungs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>8.1 ± 0.4</td>
<td>7</td>
<td>8.7 ± 0.5</td>
<td>0.7 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>CTX + apamin</td>
<td>8.6 ± 0.2</td>
<td>7</td>
<td>9.6 ± 0.3</td>
<td>1.0 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Hypertensive lungs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>23.2 ± 2.3*</td>
<td>6</td>
<td>31.0 ± 3.6*</td>
<td>7.8 ± 1.6*</td>
<td></td>
</tr>
<tr>
<td>CTX + apamin</td>
<td>25.6 ± 3.5*</td>
<td>6</td>
<td>34.6 ± 4.4*</td>
<td>9.0 ± 1.5*</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of lungs. Vehicle, saline; CTX, charybdotoxin (50 nM). The concentration of apamin used was 50 nM. *P < 0.05 vs. the respective normotensive lung value by ANOVA.
and hypertensive lungs eliminated the second hypoxic pressor response in normotensive and hypertensive lungs by adding thapsigargin (50 nM) or DMSO and 40 min after the gap junction inhibitors or vehicle. Tonic and stimulated EDHF activity. The combination of charybdotoxin plus apamin had no effect on normoxic (baseline) perfusion pressure in normotensive lungs pretreated with meclofenamate and L-NNA (Table 1). Similarly, the EDHF inhibitory cocktail did not augment the L-NNA-induced vasconstriction (38, 41) of meclofenamate-pretreated hypertensive lungs. Charybdotoxin plus apamin also had no effect on the magnitude of acute hypoxic vasoconstriction in either normotensive or hypertensive lungs (Fig. 1).

Representative perfusion pressure tracings of spontaneous (vehicle) or thapsigargin-induced vasodilation of the hypoxic pressor response in meclofenamate- and L-NNA-pretreated normotensive lungs after additional treatment with charybdotoxin plus apamin or vehicle (control) are shown in Fig. 2 and summarized in Fig. 3. The EDHF inhibitory cocktail had no effect on the spontaneous reversal of hypoxic vasoconstriction.

In contrast, whereas thapsigargin caused an immediate and marked reversal of hypoxic vasoconstriction in vehicle-control normotensive and hypertensive lungs, the PGI2- and NO-independent thapsigargin-induced vasodilation was abolished and converted to vasoconstriction in charybdotoxin plus apamin-treated lungs (Fig. 3). Role of CYP450 activity. Sulfaphenazole, but not 7-ethoxysresorufin, caused a slight increase in baseline perfusion pressure in normotensive lungs pretreated with meclofenamate and L-NNA (Table 2). In a separate group of four meclofenamate- and L-NNA-pretreated normotensive lungs, the combination of both CYP450 inhibitors did not elicit a significant increase in baseline perfusion pressure (9.4 ± 0.8 vs. 10.0 ± 1.5 mmHg, P > 0.05). In contrast, the combination of 7-ethoxysresorufin and sulfaphenazole caused a marked increase in perfusion pressure in meclofenamate- and L-NNA-pretreated hypertensive lungs (Table 2).

Table 2. Effects of ER, SP, GA, and PA on baseline perfusion pressure in meclofenamate- and N\textsuperscript{G}-nitro-L-arginine-pretreated normotensive and hypertensive lungs

<table>
<thead>
<tr>
<th>Baseline Perfusion Pressure, mmHg</th>
<th>n</th>
<th>0 min</th>
<th>15 min after treatment</th>
<th>40 min after treatment</th>
<th>ΔPressure, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Normotensive lungs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>21</td>
<td>7.7 ± 0.2</td>
<td>8.3 ± 0.2</td>
<td>8.7 ± 0.2</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>ER</td>
<td>6</td>
<td>7.8 ± 0.4</td>
<td>8.0 ± 0.5</td>
<td>8.5 ± 0.5</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>SP</td>
<td>6</td>
<td>7.0 ± 0.3</td>
<td>8.0 ± 0.4</td>
<td>8.9 ± 0.5</td>
<td>1.9 ± 0.4</td>
</tr>
<tr>
<td>GA</td>
<td>5</td>
<td>7.9 ± 0.4</td>
<td>9.0 ± 0.6</td>
<td>9.3 ± 0.4</td>
<td>1.4 ± 0.2</td>
</tr>
<tr>
<td>PA</td>
<td>8</td>
<td>7.3 ± 0.3</td>
<td>8.6 ± 0.3</td>
<td>8.9 ± 0.3</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td><strong>Hypertensive lungs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>9</td>
<td>27.5 ± 2.4</td>
<td>30.1 ± 2.7</td>
<td>28.5 ± 2.4</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>ER + SP</td>
<td>3</td>
<td>28.1 ± 0.9</td>
<td>36.1 ± 1.7</td>
<td>37.5 ± 2.4</td>
<td>9.4 ± 1.5</td>
</tr>
<tr>
<td>GA</td>
<td>6</td>
<td>28.1 ± 3.1</td>
<td>35.5 ± 5.9</td>
<td>40.5 ± 6.7</td>
<td>12.5 ± 4.2</td>
</tr>
<tr>
<td>PA</td>
<td>3</td>
<td>27.8 ± 4.4</td>
<td>33.4 ± 4.0</td>
<td>37.9 ± 6.9</td>
<td>11.9 ± 2.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of lungs. Vehicle, DMSO; ER, 7-ethoxysresorufin (3 μM); SP, sulfaphenazole (10 μM); GA, 18α-glycyrrhetinic acid (30 μM); PA, palmitoleic acid (50 μM). *P < 0.05 vs. the respective vehicle control value by ANOVA; †P < 0.05 vs. the respective other group by ANOVA. **P < 0.05 vs. the respective normotensive lung value by ANOVA.

with 3% O\textsubscript{2} for 15 min at 15 and 40 min after the addition of CYP450 inhibitors or vehicle. Stimulated EDHF activity was assessed in these lungs by adding thapsigargin (50 nM or DMSO) to the perfusate at the peak of the second hypoxic response and measuring thapsigargin-induced and spontaneous vasodilation. An additional group of normotensive lungs was treated identically except that both 3 μM 7-ethoxysresorufin and 10 μM sulfaphenazole were added to the perfusate before testing the thapsigargin-induced vasodilation.

The meclofenamate- and L-NNA-pretreated hypertensive lungs were treated only with the combination of 3 μM 7-ethoxysresorufin plus 10 μM sulfaphenazole or vehicle (DMSO). They were then challenged twice with 3% O\textsubscript{2} for 15 min at 15 and 40 min after treatment with CYP450 inhibitors or vehicle, and 50 nM thapsigargin was added to the perfusate at the peak of the second hypoxic response. In this case, the percent spontaneous and thapsigargin-induced vasodilation was measured during the first and second hypoxic response, respectively, after treatment with 18α-glycyrrhetinic acid, palmitoleic acid, or vehicle.
CYP450 inhibitors had no effect on the magnitude of acute hypoxic vasoconstriction in either normotensive or hypertensive lungs (Fig. 4).

Although neither sulfaphenazole nor 7-ethoxyresoruﬁn altered the spontaneous reversal of hypoxic vasoconstriction in meclofenamate- and L-NNA-pretreated normotensive lungs, each agent inhibited the thapsigargin-induced vasodilation (Fig. 5). In normotensive lungs treated simultaneously with both CYP450 inhibitors, thapsigargin-induced vasodilation was abolished (hypoxic perfusion pressure tended to increase 38.8 ± 14.3% within 2 min after the addition of thapsigargin) (P = 0.0825, n = 4). In contrast, the combination of CYP450 inhibitors did not inhibit the PGI2- and NO-independent thapsigargin-induced vasodilation in hypertensive lungs (Fig. 5).

Role of gap junctions. Palmitoleic acid, but not 18-glycyrrhetinic acid, caused a slight increase in baseline perfusion pressure in meclofenamate- and L-NNA-pretreated normotensive lungs (Table 2). Each of the putative gap junction blockers caused a marked increase in perfusion pressure in meclofenamate- and L-NNA-pretreated hypertensive lungs (Table 2). 18-Glycyrrhetinic acid, but not palmitoleic acid, blunted acute hypoxic vasoconstriction in both groups of lungs (Fig. 6).

Although neither agent affected the spontaneous reversal of hypoxic vasoconstriction in normotensive lungs, palmitoleic acid, but not 18-glycyrrhetinic acid, reduced the PGI2- and NO-independent thapsigargin-induced vasodilation (Fig. 7). In contrast, neither agent inhibited the thapsigargin-induced vasodilation in hypertensive lungs.

DISCUSSION

This study showed that after inhibition of both PGI2 and NO synthesis, the EDHF-inhibitory cocktail of charybdotoxin plus apamin had no effect on “baseline” perfusion pressure or acute hypoxic vasoconstriction in either normotensive or hypoxia-induced hypertensive PSS-perfused rat lungs. These findings suggested no role for tonic EDHF activity in the regulation of either normotensive or hypertensive pulmonary vascular tone. However, charybdotoxin plus apamin abolished the vasodilation induced by thapsigargin during acute hypoxic vasoconstriction in both groups of lungs. This indicated that after inhibition of PGI2 and NO synthesis, thapsigargin-induced pulmonary vasodilation was mediated by an EDHF in both normotensive and hypertensive lungs. Furthermore, the CYP450 blockers 7-ethoxyresoruﬁn and sulfaphenazole inhibited the EDHF-mediated vasodilation in normotensive lungs, suggesting the involvement of CYP450 activity in the mechanism of vasodilation. Also in normotensive lungs, there were mixed results with two different putative inhibitors of gap junction intercellular communication. Thus, palmitoleic acid, but not 18-glycyrrhetinic acid, inhibited the EDHF-mediated vasodila-
tion. Depending on whether or not palmitoleic acid was more effective than 18\textsuperscript{o}-glycyrrhetinic acid in uncoupling gap junctions (48), this finding raised the possibility that gap junction intercellular communication was involved in the EDHF-mediated pulmonary vasodilation. In contrast, neither the CYP450 inhibitors nor the gap junction blockers reduced the EDHF-mediated vasodilation in hypertensive lungs. Therefore, the mechanism of EDHF-mediated vasodilation stimulated by thapsigargin in hypertensive lungs was apparently different from that in normotensive lungs.

The finding that simultaneous treatment with inhibitors of cyclooxygenase, NOS, and EDHF caused no vasoconstriction in normoxic PSS-perfused normotensive rat lungs indicated that the low basal vascular tone in this preparation was not due to the combined actions of PGI\textsubscript{2}, NO, and EDHF. Unless another, unidentified vasodilator is responsible, it seems likely the low vascular tone is due to the absence of a vasoconstrictor signal. In contrast to the inhibition of PGI\textsubscript{2} and NO synthesis, which unmasks a largely endothelin-1-mediated vasoconstriction in hypertensive rat lungs (38, 41), inhibition of EDHF elicited no further increase in vascular tone in the hypertensive lungs. Thus tonic EDHF played no role in regulating “baseline” vascular tone in either normotensive or hypertensive rat lungs.

In contrast to the blunting of acute hypoxic pulmonary vasoconstriction by endogenously generated PGI\textsubscript{2} and NO in isolated rat lungs (28, 37, 39, 41), our results suggested no role for tonic EDHF activity in moderating hypoxic vasoconstriction in either normotensive or hypertensive rat lungs. This contradicts the speculation of Hasunuma et al. (28), based on effects of the nonselective K\textsuperscript{+} channel blocker tetraethylammonium, that EDHF moderates hypoxic vasoconstriction in normal rat lungs. Our finding differs from the report of Carter et al. (7) showing that EDHF activity is upregulated and inhibits hypoxic vasoconstriction in meclofenamate- and L-NNA-treated cirrhotic rat lungs. It should also be noted that pulmonary vascular EDHF activity and its effects on vasoreactivity might differ among different strains of rats (34).

Similar to the lack of effect of charybdotoxin plus apamin, the CYP450 inhibitors 7-ethoxyresorufin and sulfaphenazole did not affect hypoxic vasoconstriction in this study. This finding agrees with a previous
report that CYP450 metabolites do not mediate hypoxic vasoconstriction in rat lungs (8). The vasoconstriction elicited by the combination of 7-ethoxyresorufin and sulfaphenazole in meclofenamate- plus L-NNA-treated hypertensive lungs raised the possibility that a vasodilator CYP450 metabolite (59) was moderating the hypertensive vascular tone.

It is unknown whether gap junction intercellular communication plays a role in the mechanism of hypoxic pulmonary vasoconstriction. Our results showed that 18α-glycyrrhetinic acid, but not palmitoleic acid, inhibited the hypoxic response in both normotensive and hypertensive lungs. Whereas it is tempting to speculate the blunting by 18α-glycyrrhetinic acid was due to interference with hetero- and/or homocellular gap junction communication, the failure of this agent to inhibit the thapsigargin-induced, EDHF-mediated vasodilation, and its reported nonspecific effects (12, 27, 51), raise a note of caution. More direct measurements and more specific inhibitors are needed to rigorously test a possible role of gap junction intercellular communication in the mechanism of hypoxic vasoconstriction.

Comparable to receptor-dependent agonists, the Ca\(^{2+}\)-ATPase inhibitors cyclopiazonic acid and thapsigargin elicit endothelium-dependent vasodilation (35, 52, 53). After blockade of PGI\(_2\) and NO synthesis, Ca\(^{2+}\)-ATPase inhibitor-induced smooth muscle cell hyperpolarization and relaxation are attributed to EDHF. In our study, thapsigargin caused vasodilation in both normotensive and hypoxia-induced hypertensive lungs that had been pretreated with cyclooxygenase and NOS inhibitors, and this vasodilation was abolished and converted to vasoconstriction by the EDHF inhibitors charybdotoxin plus apamin. In contrast, Karamsetty et al. (34) observed that thapsigargin-induced relaxation of isolated rat intralobar pulmonary arteries was blocked by NOS inhibition, suggesting the response did not involve EDHF. A likely explanation for this difference is that whereas endothelium-dependent relaxation of conduit arteries is mediated largely by NO, EDHF activity is generally...
Fig. 7. Gap junction blocker PA (50 μM), but not GA (30 μM), reduced TG (50 nM)-induced vasodilation in meclofenamate- and N⁵-nitro-L-arginine-pre-treated NL. In contrast, neither gap junction blocker reduced the vasodilation in HL. Vehicle (DMSO, spontaneous vasodilation) or TG was added to vehicle-time control (DMSO) and gap junction blocker-treated NL and HL at peak of hypoxic (3% O₂) vasoconstriction, and vasodilation was measured 2 min later. Neither gap junction blocker altered spontaneous reversal of hypoxic vasoconstriction in either group of lungs. Values are means ± SE; numbers in parentheses represent numbers of lungs. *P < 0.05 for %TG vasodilation vs. respective %spontaneous vasodilation by ANOVA; ‡P < 0.05 for PA group vs. respective vehicle control group by ANOVA.

more important in peripheral resistance arteries (29, 30, 46, 49), which would have been responsible for the changes in perfusion pressure in the perfused lungs.

The chemical nature of EDHF has not been established, but CYP450 inhibitors have reduced EDHF-mediated vasodilation in some studies (6, 22, 45). The CYP450 1A inhibitor 7-ethoxyresorufin inhibits EDHF-mediated vasodilation in rat mesenteric (1) and renal (2) vascular beds. Similarly, inhibition of EDHF vasodilation by sulfaphenazole in the porcine coronary artery suggests a role for a cytochrome 2C isozyme (21). Various CYP450 inhibitors have been found to reduce EDHF-mediated relaxation of rat (34) and dog (31) pulmonary arteries. However, CYP450 inhibitors have not inhibited EDHF-mediated vasodilation in other studies or have done so nonspecifically (14, 18, 23, 24, 33, 36, 42, 58). In our study, 7-ethoxyresorufin and sulfaphenazole alone reduced, and the combination of inhibitors abolished, EDHF-mediated vasodilation in normotensive lungs. However, the combination of the inhibitors had no effect on the vasodilation in hypertensive lungs. This suggested that while an unidentified product (or products) of CYP450 activity was involved in the mechanism of EDHF-mediated vasodilation in normotensive rat lungs, its contribution was somehow lost or masked in hypertensive lungs.

Numerous recent studies using gap junction inhibitors suggest the contribution of gap junction intercellular communication to EDHF-mediated vasodilation. Here again, however, the results are variable. Whereas most studies show inhibition of EDHF-mediated vasodilation by the blocker 18α-glycyrrhetinic acid (10, 16, 23, 27, 29, 36, 52, 53), some do not (3, 32, 50). Similarly, palmitoleic acid inhibited EDHF responses in rat mesentery (27) and skeletal muscle (56) arteries but not in pig coronary arteries (3). In our study, palmitoleic acid, but not 18α-glycyrrhetic acid, reduced the EDHF-mediated vasodilation in normotensive lungs. We do not know why 18α-glycyrrhetic acid was ineffective. The 30 μM concentration of 18α-glycyrrhetic acid used was less than the usual 100 μM (10, 16, 27, 36, 52, 53), but higher concentrations are believed to have a variety of nonspecific effects (12, 27, 51). In addition, we found in preliminary experiments that 67 μM 18α-glycyrrhetic acid abolished hypoxic vasoconstriction and made it impossible to test thapsigargin-induced vasodilation (unpublished observations). Our results with palmitoleic acid and 18α-glycyrrhetic acid are perhaps similar to those of Schuster et al. (48) in the rat mesenteric artery, who show a much less effective inhibition of myoendothelial gap junctions by 18α-glycyrrhetic acid than by palmitoleic acid. Even so, our results are equivocal, and the role of gap junctions in the mechanism of EDHF-mediated vasodilation in rat lungs remains uncertain.

We have no explanation of why both 18α-glycyrrhetic acid and palmitoleic acid caused vasoconstriction in the meclofenamate- and l-NA-treated hypertensive lungs. The vasoconstriction was not due to the inhibition of EDHF activity, because neither agent inhibited the EDHF-mediated vasodilation, and charybdotoxin plus apamin did not elicit the response. In contrast to its vasoconstrictor effect in perfused hypertensive lungs, 18α-glycyrrhetic acid inhibits spontaneous contractions and causes relaxation of hypertensive main pulmonary arteries from chronically hypoxic rats (4).

In summary, our results in PSS-perfused rat lungs showed that although tonic EDHF activity played no role in regulating either “baseline” pulmonary vascular tone or hypoxic pulmonary vasoconstriction, thapsigargin stimulation of EDHF activity reversed acute hypoxic vasoconstriction. The mechanism of EDHF-mediated vasodilation appeared to involve CYP450 activity, and possibly gap junction intercellular communication, in normotensive lungs but apparently involved a diff
different signaling pathway in the remodeled arteries of hypoxic hypertensive lungs.

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