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Diamide Inhibits Pulmonary Vasoconstriction Induced by Hypoxia or Prostaglandin $F_{2\alpha}$ (41615)

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Abstract. Diamide oxidizes glutathione and other cellular sulfhydryl groups. It decreases calcium ATPase activity and alters mitochondrial calcium flux, probably as a result of the sulfhydryl oxidation. We examined the effect of diamide (5 mg/kg, iv) on pulmonary vascular reactivity in 12 anesthetized dogs. Diamide reversed the pulmonary vasoconstriction caused by hypoxia in seven dogs (control ΔPVR + 2.5 ± 0.6 mm Hg/liter/min; postdiamide ΔPVR − 0.1 ± 0.4 mm Hg/liter/min; $P < 0.01$). The pulmonary pressor response to prostaglandin $F_{2\alpha}$ (5 μg/kg/min, iv) was also reduced (control ΔPVR + 3.8 ± 0.5 mm Hg/liter/min; postdiamide ΔPVR + 1.1 ± 0.7 mm Hg/liter/min; $P < 0.01$). However, in a further five dogs, diamide had only a small effect on the pulmonary vasoconstriction caused by angiotensin II, while the pressor response to hypoxia was again inhibited. The mechanism by which diamide reverses pulmonary vasoconstriction is not certain but the effect is rapid, consistent, and reversible. Because the intravenous infusion of diamide does not produce systemic hypotension, during its period of action on the pulmonary vasculature, unlike the drugs currently available for the clinical treatment of pulmonary hypertension, further studies of its mechanism of action are indicated.

Diamide [diazenedicarboxylic acid bis (*N,N*-dimethylamide) II] was introduced in 1969 as an agent which could rapidly oxidize glutathione (GSH) to glutathione disulfide (GSSG) in red blood cells without otherwise altering cellular metabolism (1). Glutathione is a sulfhydryl-containing tripeptide which is present in all mammalian cells (2) and acts as a redox buffer (3). Normally almost all intracellular glutathione is maintained in the reduced state by the NADPH-requiring enzyme, glutathione reductase. When glutathione is oxidized by diamide, other sulfhydryl groups in enzymes and cell membranes are also rendered susceptible to oxidation (4). Sulfhydryl oxidation by diamide has been shown to alter $(Ca^{2+} + Mg^{2+})$-ATPase activity (5) and calcium permeability in red cell membranes (6). Because of these observations and the demonstration that diamide inhibits the spontaneous contraction of rat muscle cells in culture (7), we wished to determine whether diamide would cause pulmonary vasodilatation. During normoxia the pulmonary vasculature is almost maximally dilated and additional dilatation is difficult to discern. Consequently the hemodynamic effect of diamide was determined in anesthetized dogs when the pulmonary vascular resistance was increased by hypoxia, prostaglandin $F_{2\alpha}$, or angiotensin II. A preliminary report of part of this study has been published (8).

Materials and Methods. Twelve mongrel dogs of either sex (mean weight 24 ± 1 kg), were anesthetized with pentobarbital sodium (30 mg/kg, iv), paralyzed with succinyl choline (2 mg/kg, iv), intubated with an endotracheal tube, and ventilated on room air with a Harvard (Harvard Apparatus, 150 Dover Rd., Millis, Mass. 02054) respirator. Additional doses of pentobarbital sodium (15 mg/kg, iv) were given during the experiment to maintain anesthesia. The respirator was adjusted initially to maintain a systemic arterial pH of about 7.39. Ventilation thereafter was maintained constant throughout the experiment. Care was taken to "sigh" the dogs at regular intervals and to maintain the esophageal temperature between 38.0 and 39.5°C. Polyethylene catheters were placed in the superior and inferior vena cavae and in the descending aorta. A Swan-Ganz balloon-tipped

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catheter was advanced to a branch of the pulmonary artery. Pulmonary arterial, wedge, and aortic pressures were measured using Statham P23 ID (Statham Instruments Inc., Medical Sales Division, 2230 Statham Blvd., Oxnard, Calif. 93030) transducers and a Hewlett Packard (Hewlett Packard, Medical Electronics Division, 175 Wiman Street, Waltham, Mass. 02154) 8-channel recorder. Cardiac output was determined by injecting 1.25 mg Indocyanine-green dye into the inferior vena cava and withdrawing blood from the aorta, at 20 ml/min, through a Waters (Waters Instruments, Inc., P.O. Box 6117, Rochester, Minn. 55901) cuvette densitometer. Pulmonary vascular resistance was calculated as mean pulmonary arterial pressure minus mean wedge pressure (mm Hg) divided by cardiac output (liters/minute). Systemic arterial oxygen and carbon dioxide tensions and pH were measured with a Corning (Corning Glass Works, Medfield, Mass. 02502) Model 168 pH blood gas analyzer. In all the dogs, pulmonary and systemic hemodynamics (pressures, cardiac output, and systemic blood gases) were recorded during normoxia and after 8 and 15 min of hypoxia (inspired O_2:12%).

Seven dogs (Group 1) then had the sequence of interventions shown in Fig. 1; i.e., prostaglandin $F_{2\alpha}$ (5 μg/kg/min, iv, for 4 min), a second, third, and fourth hypoxic challenge (each of the same severity as the first), and finally a second prostaglandin $F_{2\alpha}$ infusion (Sigma Chemical Co., St. Louis, Mo. 63178). The hemodynamics were recorded before, and at the end of, each prostaglandin infusion. Diamide (5 mg/kg dissolved in 10 ml saline) was infused intravenously over 1 min, immediately after the hemodynamic recordings made at 8 min in the third hypoxic challenge, and again at the start of the second prostaglandin $F_{2\alpha}$ infusion.

Five dogs (Group 2), had the sequence of interventions shown in Fig. 3. The initial hypoxic challenge was repeated and diamide was infused after 8 min of the second challenge. Angiotensin II (0.5 μg/kg/min) (Sigma Chemical Co., St. Louis, Mo. 63178) was infused intravenously for 4 min and the hemodynamic measurements were made before and at the end of the infusion. Between all the interventions a period of 30–40 min was allowed for the hemodynamics to become stable. At the start of the second angiotensin II infusion, the diamide was given intravenously as in the case of prostaglandin $F_{2\alpha}$.

The data are given as the mean and standard error of the mean. The hemodynamic changes (Δ) produced by hypoxia, prostaglandin $F_{2\alpha}$, or angiotensin II, before and after diamide, in the same dogs, were compared by the paired t test. In those dogs exposed to several hypoxic challenges, the challenge immediately prior to the diamide infusion was used for comparison.

Results. The reduction in the inspired oxygen tension produced a reproducible increase in pulmonary arterial pressure and resistance (first hypoxic challenge: ΔP_{PA} 14 ± 1 mm Hg and ΔPVR 2.8 ± 0.5 mm Hg/liter/min; and second challenge: ΔP_{PA} 13 ± 2 mm Hg and ΔPVR 2.5 ± 0.6 mm Hg/liter/min) (Table I). The systemic arterial blood gases after 15 min of hypoxia for the two challenges were: PO_2 33 ± 1 and 32 ± 1 mm Hg; PCO_2 27 ± 1 and 27 ± 1 mm Hg; pH 7.44 ± 0.01 and 7.45 ± 0.01.

Following the intravenous injection of diamide in Group 1 dogs, the pulmonary artery pressure and pulmonary vascular resistance after 15 min of hypoxia were not significantly different from the normoxic control values (+3 ± 1 mm Hg and −0.1 ± 0.4 mm Hg/liter/min, respectively) (Figs. 1 and 2 and Table I). The systemic arterial blood gases were PO_2 34 ± 1 mm Hg, PCO_2 26 ± 1 mm Hg, and pH 7.46 ± 0.01. The same was true of Group 2 (ΔP_{PA} + 2 ± 1 mm Hg and ΔPVR + 0.3 ± 0.3 mm Hg/liter/min after 15 min of hypoxia) (Table II). In both groups, both the increase in pulmonary arterial pressure and pulmonary vascular resistance caused by hypoxia, and the absolute level of pressure and resistance attained, were reduced ($P < 0.01$) after diamide (Figs. 1–3 and Tables I and II). The systemic arterial pressure, systemic resistance, and cardiac output were unchanged. Forty minutes after diamide, the pressor response to hypoxia in Group 1 had returned to control levels (Figs. 1 and 2).

The increase in pulmonary arterial pressure and pulmonary vascular resistance stimulated by prostaglandin $F_{2\alpha}$ in Group 1 dogs (ΔP_{PA} + 10 ± 1 mm Hg and ΔPVR + 3.8 ± 0.5 mm Hg/liter/min) and the absolute levels of pressure and resistance attained were

TABLE I. DIAMIDE REDUCES THE PULMONARY PRESSOR RESPONSES TO HYPOXIA AND PROSTAGLANDIN $F_{2\alpha}$ (GROUP 1:n = 7)

	P_{AO}	P_{PA}	ΔP_{PA}	P_w	CO	SVR	PVR	ΔPVR	
Normoxia	149 ± 6	17 ± 2		4 ± 1	3.7 ± 0.3	43 ± 5	3.5 ± 0.4		
Hypoxia 8 min	172 ± 6	27 ± 2		5 ± 1	3.9 ± 0.3	46 ± 4	5.9 ± 0.5		
Hypoxia 15 min	171 ± 6	31 ± 2	14 ± 1	5 ± 1	4.2 ± 0.3	42 ± 3	6.3 ± 0.5	2.8 ± 0.5	
Pre-$F_{2\alpha}$ (control)	146 ± 5	16 ± 2		4 ± 1	3.1 ± 0.2	49 ± 4	4.0 ± 0.4		
$F_{2\alpha}$ 4 min	151 ± 5	26 ± 2	10 ± 1	4 ± 1	2.9 ± 0.1	53 ± 2	7.8 ± 0.4	3.8 ± 0.5	
Normoxia	147 ± 5	17 ± 1		4 ± 1	3.1 ± 0.2	48 ± 4	4.1 ± 0.3		
Hypoxia 8 min	165 ± 7	28 ± 2		5 ± 1	3.9 ± 0.3	45 ± 4	6.1 ± 0.6		
Hypoxia 15 min	165 ± 6	30 ± 3	13 ± 2	4 ± 1	3.9 ± 0.2	43 ± 3	6.6 ± 0.7	2.5 ± 0.6	
Normoxia	143 ± 5	15 ± 1		4 ± 1	2.8 ± 0.2	52 ± 3	3.8 ± 0.4		
Hypoxia 8 min	163 ± 5	26 ± 1		4 ± 1	3.7 ± 0.2	45 ± 3	5.8 ± 0.5		
Diamide →									
Hypoxia 15 min	161 ± 4	18 ± 1*	3 ± 1*	4 ± 1	3.8 ± 0.3	44 ± 4	3.7 ± 0.4*	−0.1 ± 0.4*	Diamide
Normoxia	143 ± 4	15 ± 1		4 ± 1	2.6 ± 0.2	50 ± 4	4.6 ± 0.6		
Hypoxia 8 min	162 ± 5	25 ± 2		5 ± 1	3.3 ± 0.3	50 ± 3	6.3 ± 0.8		
Hypoxia 15 min	162 ± 5	26 ± 2		5 ± 1	3.5 ± 0.2	47 ± 3	6.4 ± 0.8		
Pre-$F_{2\alpha}$ (control)	141 ± 3	16 ± 2		4 ± 1	2.7 ± 0.2	55 ± 4	4.7 ± 0.7		
Diamide →									
$F_{2\alpha}$ 4 min	143 ± 3	20 ± 1*	4 ± 2*	4 ± 1	2.9 ± 0.2	52 ± 3	5.8 ± 0.3*	−1.1 ± 0.7*	Diamide

Note. P_{AO}, aortic pressure (mm Hg); P_{PA}, pulmonary arterial pressure (mm Hg); P_w, pulmonary wedge pressure (mm Hg); CO, cardiac output (liter/min); SVR, systemic vascular resistance (mm Hg/liter/min); PVR, pulmonary vascular resistance (mm Hg/liter/min); $F_{2\alpha}$: prostaglandin $F_{2\alpha}$. *Different from control (prediamide) response ($P < 0.01$). Data are given as mean ± SEM.

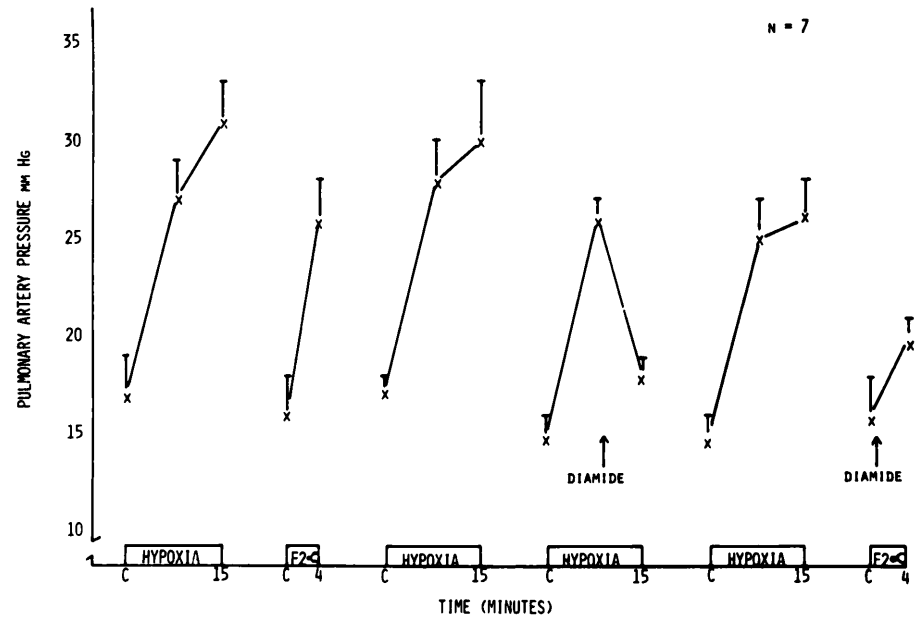

FIG. 1. The administration of diamide (5 mg/kg, iv) to seven anesthetized dogs during an hypoxic challenge (O_2:12%) or prostaglandin $F_{2\alpha}$ infusion (5 μg/kg/min, iv) reduces the pulmonary arterial pressure responses. $P < 0.01$ for the differences between the changes in pressure stimulated by hypoxia and prostaglandin $F_{2\alpha}$, before and after diamide.

reduced ($P < 0.01$) after diamide administration (Figs. 1, 2, and Table I). Prostaglandin $F_{2\alpha}$ caused a small increase in systemic vascular resistance in untreated dogs (ΔSVR $+ 4 \pm 2$ mm Hg/liter/min). However, after diamide, the infusion of $F_{2\alpha}$ was associated

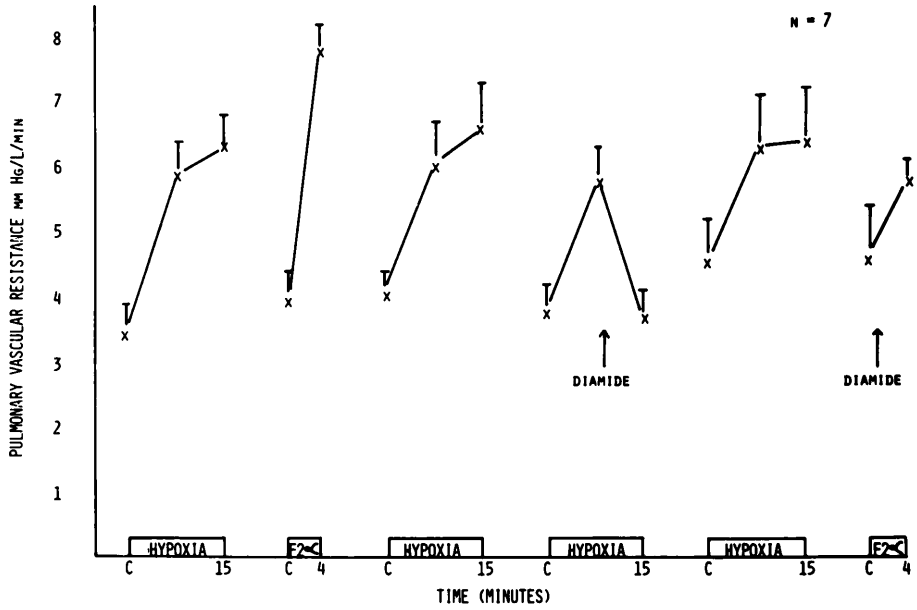

FIG. 2. Diamide (5 mg/kg, iv) reduces the pulmonary vascular resistance response to hypoxia and prostaglandin $F_{2\alpha}$. $P < 0.01$, as for Fig. 1.

TABLE II. DIAMIDE REDUCES THE PULMONARY PRESSOR RESPONSE TO HYPOXIA (GROUP 2: $n = 5$)

	P_{AO}	P_{PA}	ΔP_{PA}	P_w	CO	SVR	PVR	ΔPVR	
Normoxia	130 ± 9	11 ± 2		3 ± 1	2.4 ± 0.3	57 ± 8	3.4 ± 0.5		
Hypoxia 8 min	166 ± 9	23 ± 2		5 ± 1	2.9 ± 0.3	59 ± 5	6.1 ± 0.6		
Hypoxia 15 min	158 ± 9	24 ± 2	13 ± 2	4 ± 1	2.9 ± 0.3	57 ± 6	6.9 ± 0.5	3.5 ± 0.4	
Normoxia	130 ± 7	10 ± 2		3 ± 1	2.3 ± 0.2	58 ± 6	3.4 ± 0.8		
Hypoxia 8 min	156 ± 9	22 ± 2		4 ± 1	2.8 ± 0.2	58 ± 7	6.5 ± 0.6		
Diamide →									
Hypoxia 15 min	152 ± 6	12 ± 1*	2 ± 1*	3 ± 1	2.5 ± 0.2	64 ± 7	3.7 ± 0.5*	0.3 ± 0.3*	Diamide
Pre-AII (control)	126 ± 7	11 ± 2		3 ± 1	2.1 ± 0.1	62 ± 6	4.4 ± 0.8		
AII 4 min	186 ± 2	19 ± 1	8 ± 1	7 ± 1	2.3 ± 0.2	85 ± 9	5.4 ± 0.7	1.0 ± 0.3	
Pre-AII (control)	129 ± 5	11 ± 1		3 ± 1	2.0 ± 0.2	67 ± 6	4.6 ± 0.7		
Diamide →									
AII 4 min	182 ± 4	16 ± 1†	5± 1	8 ± 1	1.7 ± 0.2†	113 ± 14†	5.2 ± 0.7	0.6 ± 0.4	Diamide

Note. P_{AO}, aortic pressure (mm Hg); P_{PA}, pulmonary arterial pressure (mm Hg); P_w, pulmonary wedge pressure (mm Hg); CO, cardiac output (liter/min); SVR, systemic vascular resistance (mm Hg/liter/min); PVR, pulmonary vascular resistance (mm Hg/liter/min); AII, angiotensin II. *Different from control (prediamide) response $P < 0.01$. †Different from control (prediamide) response $P < 0.05$. Data is given as mean ± SEM.

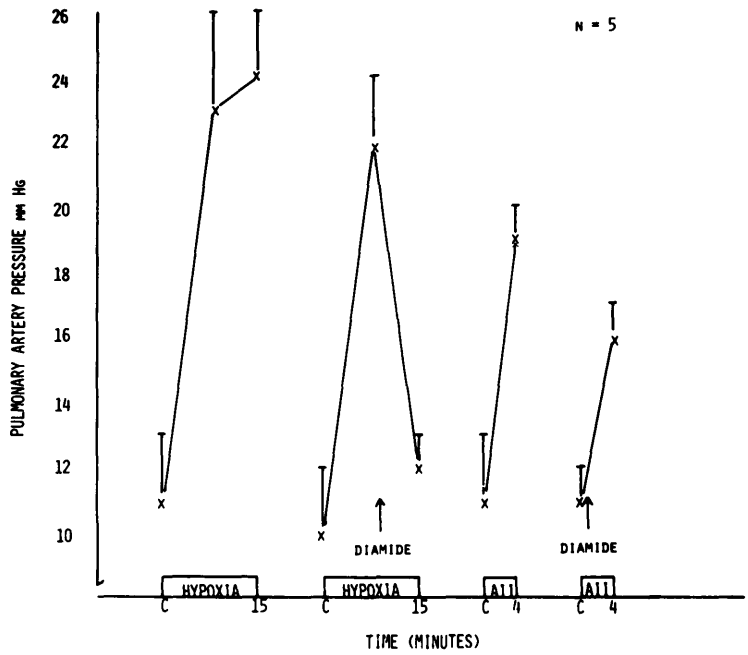

FIG. 3. The administration of diamide (5 mg/kg, iv) to five anesthetized dogs during an hypoxic challenge (O_2:12%) or angiotensin II infusion (0.5 μg/kg/min) reduces the pulmonary arterial pressure responses. $P < 0.01$ for the difference between the changes in pressure stimulated by 15 min of hypoxia, before and after diamide. $P < 0.05$ for the difference between the changes in pressure stimulated by angiotensin II, before and after diamide.

with a small decrease in systemic resistance (ΔSVR $-$ 3 $\pm$ 2 mm Hg/liter/min) ($P < 0.05$).

The modest increase in pulmonary arterial pressure (ΔP_{PA} + 8 mm Hg) caused by angiotensin II was slightly reduced by diamide in all dogs of Group 2 (ΔP_{PA} + 5 mm Hg; $P < 0.01$) (Fig. 3 and Table II). However, infusion of angiotensin II after diamide was also associated with a small fall in cardiac output. Consequently the calculated pulmonary vascular resistance response to angiotensin II did not change after diamide (Table II).

Discussion. The hemodynamic data demonstrate that diamide temporarily reverses the pulmonary hypertension observed during alveolar hypoxia. It also reduces the pulmonary vascular pressor response to prostaglandin $F_{2\alpha}$ and induces a small decline in the pulmonary arterial pressure response to angiotensin II. The fact that diamide prevents hypoxic pulmonary hypertension is of interest because of the potential therapeutic implications and the possibility that it may help to elucidate the mechanism of hypoxic pulmonary vasoconstriction.

A selective pulmonary vasodilator is not available for clinical use in patients with pulmonary hypertension. As a result, the administration of vasodilator agents in the treatment of pulmonary hypertension often causes systemic hypotension. In this study the intravenous administration of diamide markedly decreased pulmonary vasoconstriction during hypoxia without changing cardiac output, aortic pressure, or systemic vascular resistance. Consequently it would be of interest to elucidate the mechanism by which diamide has a selective effect in this acute model. The observation that the activity of diamide was limited to the pulmonary vasculature could be due to a specificity of the agent for pulmonary arteriolar smooth muscle, or to the fact that it reached the pulmonary bed before the systemic. In either case, a selective action of this sort would be helpful in the management of patients with pulmonary hypertension. Clearly, the finding that diamide causes pulmonary vasodilatation in the anesthetized dog does not mean that it would necessarily be effective in patients with chronic pulmo-

nary hypertension, but suggests that further studies of its mechanism of action are warranted. Experimental animal studies on the pulmonary and systemic hemodynamic actions and the side effects of prolonged infusions of diamide, or a longer-acting analogue, would be necessary before any consideration could be given to clinical use.

The mechanism by which diamide produces pulmonary vasodilatation is not known, but several possibilities exist. (i) Acetylcholine has long been known to cause pulmonary vasodilatation in the presence of pulmonary hypertension (9). Diamide increases acetylcholine release at neuromuscular junctions, probably by alteration of membrane protein sulfhydryl groups (10), and consequently a higher local concentration of acetylcholine is one potential mechanism for the vasodilatation induced by diamide. (ii) Transmembrane calcium flux is necessary for pulmonary vasoconstriction during hypoxia (11). The sulfhydryl groups of calcium ATPase in the sarcoplasmic reticulum of skeletal muscle are essential for its transport function (12, 13). Diamide-induced oxidation of sulfhydryl groups in calcium–magnesium ATPase decreases the ATPase activity, while reduction of the disulfides restores activity (5). The role of ATPase activity in the mechanism of hypoxic vasoconstriction is unknown, but the inhibition of that activity by diamide and the reversal by diamide of hypoxic pulmonary vasoconstriction, suggest that further studies may be appropriate to explore the possible association. (iii) The reduction of glutathione disulfide (GSSG) to glutathione (GSH) results in the consumption of NADPH. *In vitro* oxidation of such pyridine nucleotides by diamide (14) and other agents (15, 16) alters membrane permeability and calcium flux in mitochondria from liver and heart. Whether diamide alters calcium transport in pulmonary vascular smooth muscle mitochondria, sarcoplasmic reticulum, or cell membranes is unknown, but might constitute the mechanism of diamide-induced vasodilatation. One additional experiment makes it seem plausible that sulfhydryl groups may be involved, whether in glutathione or elsewhere. Although diamide, which oxidizes sulfhydryl groups, causes pulmonary vasodilatation, substances that alkylate sulfhydryl groups cause pulmonary hypertension (17).

Whatever the means by which diamide causes pulmonary vasodilatation, the agent is remarkable for its speed of action, consistent effect, and the absence of a systemic hemodynamic change. The pulmonary vasodilatation induced by diamide was seen to almost the same extent during prostaglandin $F_{2\alpha}$ infusion. This lack of specificity does not indicate whether diamide is causing vasodilatation by the same mechanism as oxygen, in view of the fact that increasing alveolar oxygen tension also markedly diminishes the pulmonary vascular pressor response to prostaglandin $F_{2\alpha}$ (18).

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