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# Amrinone Dilates Pulmonary Vessels and Blunts Hypoxic Vasoconstriction in Isolated Rat Lungs (41632)

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*Abstract.* The direct effects of the cardiotonic agent amrinone (WIN 40680) on pulmonary vasoreactivity were studied in isolated, blood-perfused, rat lungs. Lungs were perfused at constant flow and ventilated with normoxic gas, while mean pulmonary arterial pressure was monitored. Pressor responses to hypoxic gas (3% $O_2$, 5% $CO_2$) and angiotensin II (0.25 μg/0.1 ml) were elicited after amrinone or saline was added to the perfusate. Pressor responses were blunted when calculated amrinone perfusate concentration was increased to 10 μg/ml. Pressor responses to hypoxia fell from 8 ± 1 mm Hg (mean ± SE, $n$ = 8) before, to 2 ± 1 mm Hg after amrinone (10 μg/ml), while responses to angiotensin II fell from 7 ± 1 to 3 ± 1 mm Hg ($n$ = 8). Pressor responses in saline controls did not change. Pulmonary arterial pressure dropped within minutes following injection of amrinone (300 μg) into the pulmonary arterial cannula, with a greater drop during hypoxia than during normoxia. Meclofenamate did not prevent blunting of pressor responses by amrinone. Amrinone is a rapidly acting vasodilator which blunts vasoconstriction due to hypoxia or angiotensin II. Since it combines cardiotonic and pulmonary vasodilator actions, amrinone may have therapeutic potential for patients with cor pulmonale and pulmonary hypertension.

Amrinone (5-amino-3,4′-bipyridin-6(1H)-one), a recently synthesized cardiotonic agent, increases inotropy in normal and failing animal hearts (1). Recent studies in patients with congestive heart failure show increased cardiac contractility and left ventricular ejection fraction, lowered ventricular filling pressure, and decreased peripheral as well as pulmonary vascular resistances following both acute and intravenous (2, 3) and oral (4) administration of amrinone. Although investigations on the direct action of amrinone on systemic vessels demonstrate a nonspecific relaxant effect (5, 6), studies on the direct action of amrinone on pulmonary vessels are lacking. We sought to determine whether the decreased pulmonary vascular resistance following amrinone administration is due to increased cardiac output, or to a direct vasodilator effect. Demonstration of a direct vasodilator effect, in combination with its known cardiotonic action, would suggest a therapeutic role for amrinone, in patients with pulmonary hypertension and cor pulmonale.

We assessed the effects of amrinone on pulmonary arterial pressure and pressor responses to hypoxia and angiotensin II using isolated rat lungs, perfused at constant flow rates. The findings demonstrate amrinone to be a rapidly acting pulmonary vasodilator which also inhibits hypoxic and angiotensin II-induced vasoconstriction.

**Materials and Methods.** Lungs from male, 250- to 300-g, Sprague–Dawley rats were isolated and perfused with 30 ml heparinized whole blood from other rats. The procedure for isolation and perfusion has been described in detail elsewhere (7). Briefly, rats were anesthetized with pentobarbitol (25 mg/kg, ip), and the trachea, pulmonary artery, and left ventricle were cannulated. The lungs and heart were removed *en bloc* and perfused with heparinized blood from other rats at a constant flow of 0.03 ml/gm body weight/min while mean pulmonary arterial pressure was monitored. Lungs were ventilated with a humid mixture of 5% $CO_2$–95% air (normoxic gas) at 64 breaths per minute using 10 cm $H_2O$ positive inspiratory pressure and 2.5 cm $H_2O$ positive end-expiratory pressure. Perfusate temperature was maintained at 37°C. Left ventricular effluent blood was sampled intermittently for blood gas determinations using Instrumentation Laboratory microelectrodes. Blood pH was kept between 7.30 and 7.40 by additions of $NaHCO_3$ or HCl to perfusate.

After a 20-min equilibration period, pressor responses (the rise in mean pulmonary



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arterial pressure from baseline to peak) were elicited by alternating challenges every 15 to 25 min with hypoxic gas (3% $O_2$, 5% $CO_2$, 92% $N_2$) and intraarterial injections of angiotensin II (0.25 μg) during normoxia, allowing mean pressure to return to baseline prior to the next pressor response. Exposures to hypoxia were sufficiently long to allow development of a maximal pressor response, usually 10 to 12 min. Blood pH and gas tensions were pH 7.40 ± 0.01, $PO_2$ 155 ± 4 mm Hg, and $PCO_2$ 40 ± 1 mm Hg ($n$ = 12) during normoxia and pH 7.36 ± 0.01, $PO_2$ 39 ± 2 mm Hg, and $PCO_2$ 37 ± 1 mm Hg ($n$ = 12) during hypoxia (mean ± SEM). Blood gas tensions during normoxia and hypoxia did not differ between experimental groups.

As has been noted by others (8) pressor responses to hypoxia reached a maximum after three to four exposures, and then began to decline slightly. Accordingly, we administered amrinone only during or after the third hypoxic pressor response, when a maximal response had been obtained.

The first series of experiments assessed the effect of increasing concentrations of amrinone on pressor responses to hypoxia and angiotensin II. Concentrations were based on those found to increase myocardial contractility *in vitro* (1), and were calculated by dividing the amount of amrinone administered by the perfusate volume. Amrinone, or saline alone in control rats, was added to the blood reservoir during normoxia 10 min after the third, fourth, and fifth angiotensin II responses and 10 min prior to the fourth, fifth, and sixth hypoxic pressor responses. Calculated amrinone concentrations increased to 1 μg/ml after the first addition (dose 30 μg), 10 μg/ml after the second (dose 270 μg), and 100 μg/ml after the third (dose 2.7 mg). Following injection of amrinone or saline, we assessed the effect of amrinone on the subsequent hypoxic and angiotensin II pressor responses, and on baseline perfusion pressures.

In a second series of experiments, amrinone (300 μg), or saline, was injected into the pulmonary artery cannula 12 min after initiation of the fourth challenge with hypoxia, after attainment of a maximal hypoxic pressor response. Hypoxic ventilation was continued for an additional 10 min after amrinone injection, when normoxic ventilation was resumed. This allowed assessment of amrinone's vasodilator action during hypoxic vasoconstriction.

In another two experiments, the duration of action of amrinone was tested by repeated alternation of hypoxic and angiotensin II pressor responses at 10 min intervals for 70 min after attainment of a 10 μg/ml amrinone concentration.

Because recent evidence suggests a role for vasodilator prostaglandin release in the action of certain pulmonary vasodilators (13), we sought to determine whether similar release might contribute to the effects of amrinone. Meclofenamate, in amounts calculated to achieve a concentration of 20 μg/ml, or saline alone, was added to the perfusate 10 min following the third hypoxic and angiotensin II pressor responses in another series of experiments. Hypoxic and angiotensin II pressor responses were tested again after meclofenamate was added, and after each of three amrinone additions to the perfusate (final concentrations 10, 60, and 100 μg/ml). As in the first series of experiments, amrinone was added to the perfusate 10 min after the previous angiotensin II, and 10 min before the subsequent hypoxic pressor response.

Amrinone was a gift from the Sterling–Winthrop Research Institute, Rensselaer, New York and was dissolved in normal saline at concentrations of 5 mg/ml or 0.5 mg/ml. Sodium meclofenamate was a gift of the Warner–Lambert Company, Ann Arbor, Michigan. Angiotensin II was obtained from Sigma Chemical Company.

Data are expressed as means ± SE. Statistical comparisons for pressor responses between experimental and control lungs at various amrinone concentrations were performed using Student's $t$ test. Comparisons between successive pressor responses within each group of lungs were performed using one-way analysis of variance and the Student–Newman–Keuls test (9). Differences were considered significant when $P < 0.05$.

**Results.** *Blunting effect of increasing amrinone doses on hypoxic and angiotensin II pressor responses.* Figure 1 shows successive hypoxic (upper panel) and angiotensin II (lower panel) pressor responses in eight amrinone-treated and eight saline control lungs at different perfusate amrinone concentra-

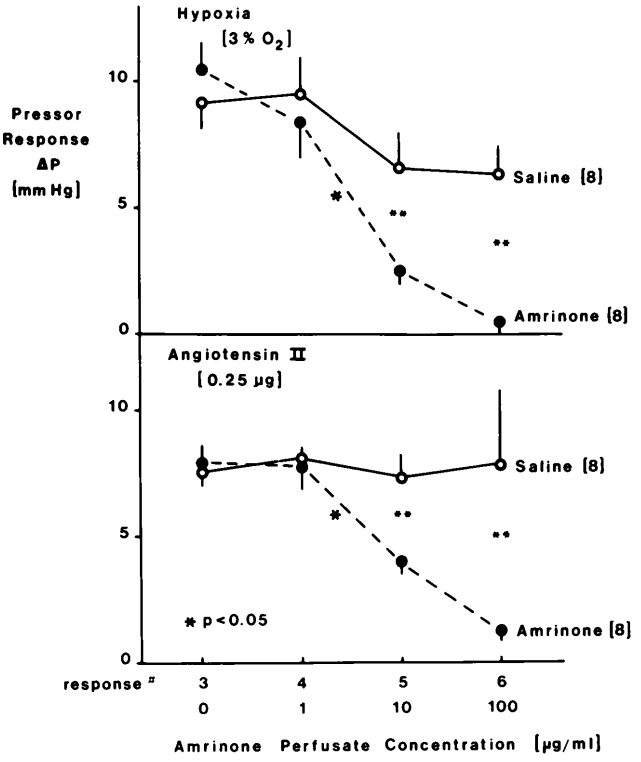


FIG. 1. The effect of increasing amrinone perfusate concentrations on successive pressor responses to hypoxia (3% $O_2$—upper panel) and angiotensin II (0.25 μg—lower panel) is shown. When amrinone concentration was increased to 10 μg/ml, pressor responses were blunted, both with respect to the contemporaneous ones in the saline group (open circles), as well as the previous ones in the amrinone group (closed circles). Further blunting occurred with 100 μg/ml amrinone. Parentheses indicate *n* in each group. Data are mean ± SE. (*) Indicates significant difference ($P < 0.05$) between successive pressor responses in the same group. (**) Indicates significant difference ($P < 0.05$) between mean pressor responses of amrinone and control groups.

tions. The third and fourth pressor responses were the same in both amrinone and control groups prior to and after addition of amrinone, 1 μg/ml, to the perfusate. When perfusate amrinone concentration was increased to 10 μg/ml, however, both hypoxic and angiotensin II pressor responses were blunted in the amrinone group compared to the previous pressor responses (single asterisks) as well as to contemporaneous pressor responses in saline controls (double asterisks). Further blunting occurred in the amrinone group when perfusate amrinone concentration was increased to 100 μg/ml. In two experiments, blunting of pressor responses persisted unchanged following an increase in amrinone concentration to 10 μg/ml until the experiments were terminated 70 min later. This suggests that the greater blunting at the 100 μg/ml concentration was not due to passage of time alone and that the effects of amrinone were not reversed in this length of time.

Table 1 shows baseline perfusion pressures prior to each of the hypoxic and angiotensin II pressor responses in Fig. 1. Baseline pressures fell slightly in the amrinone group after additions of amrinone, while baseline pressures in the saline group rose gradually. The difference between the two pressures was statistically significant before the sixth angiotensin II pressor response. It appears unlikely that baseline pressure differences were responsible for the blunting effect of amrinone because baseline pressures were nearly equal before the fifth pressor responses, when blunting first occurred.

TABLE I. BASELINE PERFUSION PRESSURES PRIOR TO SUCCESSIVE HYPOXIC AND ANGIOTENSIN II PRESSOR RESPONSES IN AMRINONE AND SALINE LUNGS

| | | Amrinone perfusate concentrations | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 0 μg/ml [3] | | 1 μg/ml [4] | | 10 μg/ml [5] | | 100 μg/ml [6] | |
| | *n* | H[a] | AII[b] | H | AII | H | AII | H | AII |
| Amrinone | 8 | 14 ± 1 | 14 ± 1 | 14 ± 1 | 15 ± 1 | 13 ± 1 | 13 ± 1 | 12 ± 1 | 12 ± 1[c] |
| Saline | 8 | 13 ± 1 | 14 ± 1 | 14 ± 1 | 15 ± 1 | 14 ± 1 | 16 ± 1 | 15 ± 2 | 17 ± 2 |

*Note.* Response numbers are in brackets.
[a] Baseline perfusion pressure (mean ± SE, mm Hg) prior to hypoxic (3% $O_2$) challenge.
[b] Baseline perfusion pressure (mean ± SE, mm Hg) prior to angiotensin II (0.25 μg) challenge.
[c] Difference between means of amrinone and saline lungs is statistically significant, $P < 0.05$.

*Vasodilation by amrinone in hypoxic and normoxic lungs.* Figure 2 shows the effect on mean pulmonary arterial pressure of amrinone (300 μg = 10 μg/ml perfusate concentration) injected into the pulmonary arterial cannula following attainment of a maximal pressor response during hypoxia. Mean pulmonary arterial pressure fell abruptly in the arminone-treated lungs, and continued to decline gradually for the next 10 min, when normoxic ventilation was resumed. There was also a slight decline in mean pulmonary arterial pressure following an intraarterial injection of saline (0.06 ml) in the control group, which may be due, in part, to decreased perfusate viscosity, as well as to a spontaneous decline as previously noted by Tucker and Reeves (10). The greater drop in mean pulmonary arterial pressure in the amrinone as opposed to the saline group became statistically significant 3 min following injection. The mean decrease in mean pulmonary arterial pressure

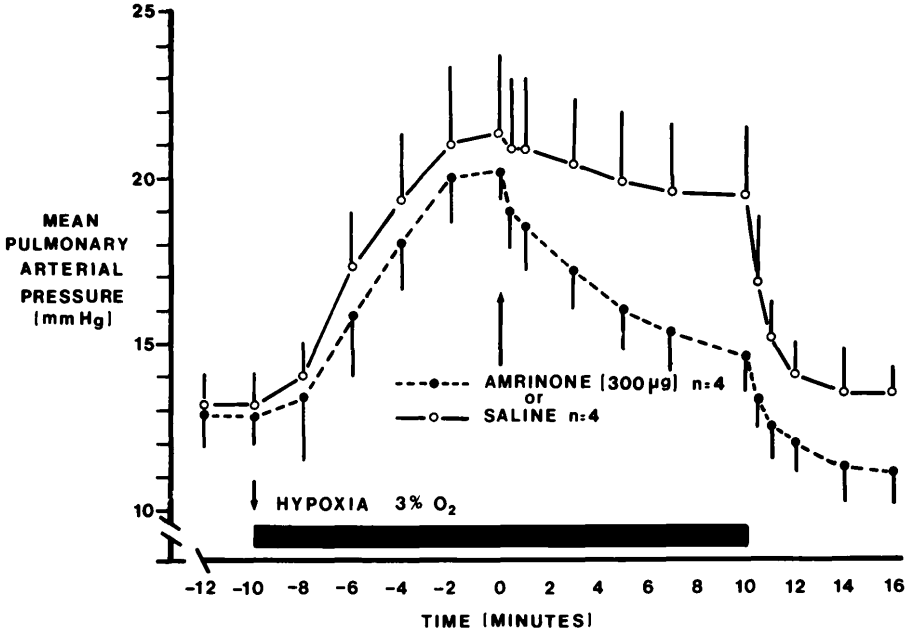


FIG. 2. Mean pulmonary arterial pressure is shown during the development of hypoxic pressor responses in amrinone and saline groups, and for 10 min following intraarterial injection of amrinone or saline, after which normoxic ventilation was resumed. Mean pulmonary arterial pressure fell more rapidly following amrinone than following saline injection. Upgoing arrow indicates time of injection of amrinone or saline (0 min). Solid bar indicates period of hypoxic ventilation. Data are mean ± SE.

10 min following injection was 5.6 ± 0.5 mm Hg in the amrinone group and 1.7 ± 0.3 mm Hg in the saline group ($P < 0.05$, $n = 4$ in each group). Thus, arminone dilated, within minutes, pulmonary vessels previously constricted by hypoxia.

During normoxia, amrinone added to the perfusate (10 μg/ml) lowered mean pulmonary arterial pressure 1.7 ± 0.03 mm Hg over the subsequent 10 min, while mean pressure in controls receiving saline fell 0.3 ± 0.2 mm Hg; ($P < 0.05$, $n = 8$ in each group). This suggests that amrinone dilates vessels under normoxic, resting conditions, as well. The greater drop in pressure following amrinone during hypoxia undoubtedly reflects the higher vessel tone brought about by hypoxic vasoconstriction and thus greater potential for dilatation.

*Effect of meclofenamate on amrinone blunting of hypoxic and angiotensin II pressor responses.* Figure 3 compares amrinone blunt-

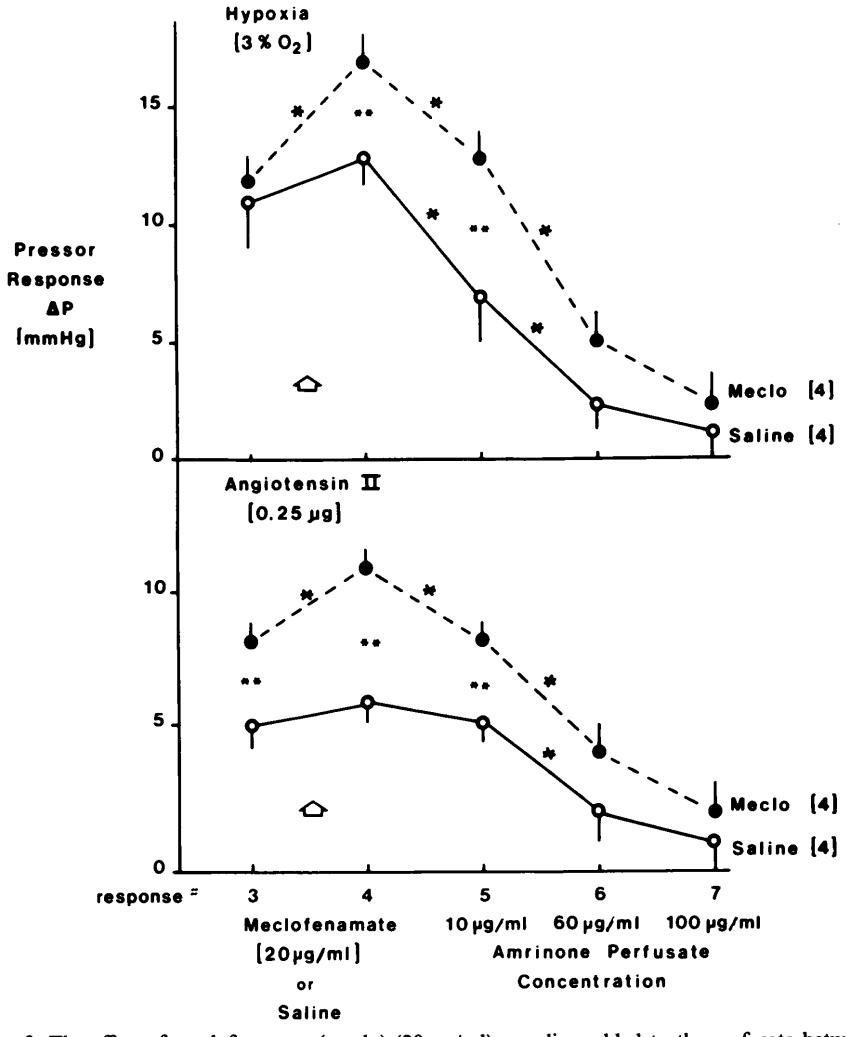


FIG. 3. The effect of meclofenamate (meclo) (20 μg/ml) or saline added to the perfusate between the third and fourth pressor response (open arrows) on subsequent arminone blunting is assessed. The fourth pressor responses were augmented by meclofenamate, but subsequent pressor responses were blunted as amrinone perfusate concentrations were increased in both saline (open circles) and meclofenamate (closed circles) groups. Parentheses indicate $n$ in each group. Data are mean ± SE. (*) Indicates significant difference ($P < 0.05$) between successive pressor responses in the same group. (**) Indicates significant difference ($P < 0.05$) between mean pressor responses of amrinone and control groups.

ing of hypoxic and angiotensin II pressor responses in lungs given meclofenamate (perfusate concentration 20 μg/ml) to that in control lungs given saline alone. As has been observed by others (8), meclofenamate augmented subsequent hypoxic and angiotensin II pressor responses, while those in saline controls remained essentially unchanged between the third and fourth responses. Addition of amrinone to the perfusate blunted hypoxic and angiotensin II pressor responses in both the meclofenamate-treated and saline control groups of lungs. Although pressor responses in the meclofenamate group remained greater than those in the saline group at the 10 μg/ml amrinone perfusate concentration, (double asterisks), amrinone still caused significant decrements in pressor responses (single asterisks) despite the presence of meclofenamate. The 100 μg/ml amrinone concentration markedly blunted pressor responses in both groups of lungs and abolished differences between the groups.

Table II shows baseline perfusion pressures prior to the hypoxic and angiotensin II pressor responses displayed in Fig. 3. Although no statistically significant differences occurred, pressures in the meclofenamate group showed a tendency to rise following addition of meclofenamate to the perfusate, and then fell to the same level as in the saline control group following addition of amrinone.

**Discussion.** The cardiotonic action of amrinone in normal and failing hearts is well documented (1, 11). In patients with congestive heart failure, amrinone improves cardiac output following intravenous (2, 3) as well as oral administration (4). Less information is available, however, on the direct action of amrinone on vascular smooth muscle. Hemodynamic studies in congestive heart failure patients have shown lowered systemic and pulmonary vascular resistances after amrinone, (2, 3) although these changes could reflect passive distension due to increased cardiac output rather than active vasodilation. In studies on isolated systemic vascular rings, amrinone acts as a nonspecific smooth muscle relaxant (5, 6).

Our results show that in isolated, perfused, rat lungs, amrinone dilates pulmonary vessels during normoxia and hypoxia and blunts pressor responses to hypoxia, and angiotensin II. Following intraarterial injection, it dilates vessels of hypoxic lungs within minutes, the effect persisting for at least 1 hr. The calculated concentration of amrinone which dilated pulmonary vasculature in our study, 10 μg/ml or greater, is similar to concentrations which increase myocardial contractility *in vitro* (1, 11). Therapeutic blood levels from human studies have not been reported, but it is likely that levels effective in our study are similar to those attained following intravenous administration in humans (2).

The mechanism of amrinone action, either with regard to increasing cardiac contractility, or relaxing smooth muscle, remains unclear. Studies on vascular and intestinal smooth muscle *in vitro,* implicate decreased calcium influx into cells and increased cyclic AMP concentrations in the mechanism of action of amrinone (5). Other investigators have reported that amrinone blocks contractions of

TABLE II. BASELINE PERFUSION PRESSURES PRIOR TO HYPOXIC AND ANGIOTENSIN II PRESSOR RESPONSES IN LUNGS GIVEN MECLOFENAMATE OR SALINE BEFORE AMRINONE

| | | | | | | Amrinone perfusate concentrations | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | [3] | | [4] | | 10 μg/ml [5] | | 60 μg/ml [6] | | 100 μg/ml [7] | |
| | *n* | H[a] | AII[b] | H | AII | H | AII | H | AII | H | AII |
| Meclofenamate (20 μg/ml) | 4 | 16 ± 1 | 16 ± 1 | 16 ± 1 | 18 ± 1 | 17 ± 2 | 18 ± 2 | 14 ± 1 | 14 ± 1 | 14 ± 1 | 12 ± 1 |
| Saline | 4 | 15 ± 1 | 15 ± 1 | 15 ± 1 | 15 ± 1 | 14 ± 1 | 14 ± 1 | 13 ± 1 | 12 ± 1 | 12 ± 1 | 12 ± 1 |

*Note.* Response numbers are in brackets.

[a] Baseline perfusion pressures (mean ± SE, mm Hg) prior to hypoxic (3% $O_2$) challenge.

[b] Baseline perfusion pressure (mean ± SE, mm Hg) prior to angiotensin II (0.25 μg) challenge.

isolated systemic vascular rings which are dependent on release of bound intracellular calcium (6). Thus, the mechanism of action of amrinone on systemic vascular smooth muscle is not clear, but the drug may inhibit contractility by several effects that decrease the availability of calcium for activation–contraction coupling.

Inhibitors of calcium influx into cells, such as verapamil also blunt hypoxic and angiotensin II vasoconstriction in isolated, perfused rat lungs (7). It is possible that the pulmonary vascular effects of amrinone are also due to inhibition of calcium uptake into cells. However, unlike verapamil which selectively blunts hypoxia more than angiotensin II pressor responses, amrinone blunted these pressor responses equally in the present study. Despite some similarities in action upon calcium movements in vascular smooth muscle, the lesser selectivity in blunting pressor responses in our experiments suggests that amrinone differs from other blockers of calcium influx in its mechanism of pulmonary vasodilation.

Vasodilator prostaglandins have been implicated as mediators in the action of some pulmonary vasodilator drugs. Nitroglycerin stimulated the release of $PGI_2$, a potent pulmonary vasodilator, by human umbilical-cord vein endothelial cells in tissue cultures (12). In addition, inhibitors of cyclo-oxygenase prevented blunting by hydralazine of hypoxic pulmonary vasoconstriction in dogs (13), suggesting that hydralazine blunted vasoconstriction through release of vasodilator prostaglandins. To determine if prostaglandin synthesis inhibitors might similarly prevent blunting of hypoxic vasoconstriction by amrinone, we added meclofenamate, an inhibitor of cyclo-oxygenase, to the blood perfusate of isolated rat lungs prior to additions of amrinone.

Although we did not measure prostaglandin levels, it is likely that meclofenamate inhibited cyclo-oxygenase activity, because the pressor responses immediately following meclofenamate addition were augmented. Others have used similar meclofenamate concentrations to inhibit cyclo-oxygenase activity in isolated, blood perfused rat lungs and have observed a progressive increase in serial pressor responses following meclofenamate addition to the perfusate (8). In our study, meclofenamate did not prevent amrinone blunting of pulmonary pressor responses, and we conclude that a cyclo-oxygenase product is not responsible for amrinone's effects on the lung vasculature.

This study demonstrates that amrinone is a rapidly acting nonspecific pulmonary vasodilator in isolated, perfused rat lungs. In combination with its known cardiotonic action, this property makes amrinone potentially useful in certain clinical situations. Unlike digoxin, which often raises pulmonary vascular resistance while increasing myocardial contractility (14), amrinone might reduce right ventricular afterload and improve cardiac function in patients with decompensated cor pulmonale, while avoiding the tachycardia and arrhythmogenicity associated with beta-sympathomimetics. In addition, certain patients with severe idiopathic pulmonary hypertension who develop systemic hypotension following use of negative inotropic agents such as calcium channel blockers, might better tolerate amrinone.

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