Pulmonary Vascular Responses to Hypercalcemia and Hypocalcemia in the Dog


The pulmonary artery responses in the isolated whole-blood perfused canine lung to ionized calcium ([Ca++]^i) were quantified over a range of hypercalcemia and hypocalcemia values ([Ca++]^i) = 0.23–1.86 mmol/L) under conditions of controlled pulmonary blood flow and constant mean aortic and left atrial pressures. Calcium chloride, administered as bolus doses in the clinical range (5–15 mg·kg^−1) at initial normocalcemia and without interventions producing vasoconstriction did not influence the slope of the pulmonary artery pressure–flow plot. Stable hypercalcemia ([Ca++]^i) = 1.86 ± 0.05 mmol/L) did not influence the slope of the pulmonary artery pressure–flow plot. Because normal pulmonary vasomotor tone is low and cannot readily be lowered further, the possible vasodilator action of hypocalcemia was assessed by its ability to decrease the slope of the mean pulmonary artery pressure–flow plot, which had been increased by alveolar hypoxia (AHX) or infusion of the prostaglandin endoperoxide analog U46619 (PG). During AHX (n = 8), a graded reduction from normocalcemia ([Ca++]^i) = 1.08 ± 0.02 mmol/L) to moderate hypocalcemia ([Ca++]^i) = 0.8 and 0.5 mmol/L did alter the pulmonary artery pressure–flow plot, but severe hypocalcemia ([Ca++]^i) = 0.26 ± 0.01 mmol/L) decreased the slope by 13 ± 0.9 mmHg·L^−1·min^−1. The comparison of severe hypocalcemia ([Ca++]^i) = 0.23–0.27 mmol/L) vs a high dose of nifedipine (bolus of 10 µg/kg followed by continuous infusion at 40 µg·kg^−1·h^−1 on pulmonary vascular tone increased by either AHX or PG infusion indicated that both hypocalcemia and nifedipine decreased the slope of the relationship between mean pulmonary artery pressure and flow (during AHX: 16:1.1 ± 1.38 and 23.3 ± 1.75 mmHg·L^−1·min^−1, both P = 0.0001 vs. AHX alone, and during PG: 17.05 ± 1.96 and 8.4 ± 1.78 mmHg·L^−1·min^−1, P = 0.0001 vs. PG alone). Two principal conclusions emerge. First, the pulmonary vessels are minimally sensitive to changes in ionized calcium throughout the clinical hypercalcemia and hypocalcemia ranges; extreme hypocalcemia is required to produce vasodilation, which was reversed with calcium infusion. Second, whereas the pulmonary vasodilator effects of extreme hypocalcemia were independent of the intervention inducing pulmonary vasoconstriction (AHX vs. PG), those of nifedipine were much more pronounced with AHX. (Key words: Ionic calcium; Lungs; pulmonary vasomotion; constriction; dilation. Pharmacology, calcium channel-blocking drugs; nifedipine.)

The calcium ion concentration ([Ca++]^i) in the blood is a major determinant of vascular smooth muscle contractile force. Constriction of vascular smooth muscle requires increased cytosolic calcium ion, made possible by calcium influx into the cell interior. In the pulmonary vessels, smooth muscle is primarily located in the walls of small pulmonary arteries.

We undertook this study to answer three principal questions. First, what is the pulmonary artery response to calcium infusion? This question is of clinical importance as calcium is infused in patients during anesthesia and in the critical care setting. Although isolated rat lung experiments have shown that the pulmonary vessels contract at unphysiologically high calcium levels (total calcium ranging from 9 to 10 µmol/L, which is nearly four times the normal value), the pulmonary artery response to acute hypercalcemia in the therapeutic range is unknown. Therefore, we studied the pulmonary artery response to physiologic levels of hypercalcemia in the canine lung.

Second, what is the pulmonary artery response to hypocalcemia? Although hypocalcemia is a peripheral vasodilator, its pulmonary vascular effects cannot be predicted from effects in another vascular bed.

Third, if hypocalcemia is a pulmonary vasodilator, what degree of hypocalcemia is required to produce pulmonary vasodilation similar to that produced by a dose of nifedipine in the high-dose range and known to have a substantial pulmonary vasoactive effect? There is strong evidence to support the concept of calcium flux as essential to diverse stimuli known to modulate pulmonary vasomotion. For example, both in the experimental animal and in humans pulmonary vasomotor tone is reduced by drugs, such as nifedipine, that inhibit calcium ion flux via calcium channels. In contrast, pulmonary vasomotor tone is increased by specific calcium channel potentiotators. Chemically diverse humoral agonists acting through receptors in smooth muscle are also dependent, at least in part, on calcium flux, including norepinephrine, serotonin, angiotensin II, and products of the arachidonic acid cascade.

In the dog, resting pulmonary vascular tone is low and cannot readily be lowered further, as demonstrated with the potent vasodilator sodium nitroprusside. In contrast, pulmonary vasodilation by organic nitrates may be readily demonstrated when pulmonary vascular tone is increased before their administration. Similarly, other canine and human studies have shown the calcium channel blocking drug nifedipine to be a potent pulmonary vasodilator by its ability to inhibit hypoxic pulmonary va-
However, its ability to reverse pulmonary vasoconstriction due to prostaglandin compounds is weak.\textsuperscript{6,7,17} These considerations contributed to the protocols designed to answer questions 2 and 3. To test the hypothesis that hypocalcemia has pulmonary vasodilator action, in one group of dogs we first produced vasoconstriction secondary to alveolar hypoxia and we lowered ionized calcium until reversal of pulmonary vasoconstriction was apparent. In other dogs, we compared the effects of hypocalcemia and nifedipine. In one group of dogs, we produced alveolar hypoxia and lowered ionized calcium in pulmonary artery blood to a level to produce effects similar to those of the selected dose of nifedipine. In another group, we tested the same level of hypocalcemia and dose of nifedipine in their ability to reverse pulmonary vasoconstriction produced by another vasoconstrictor, U46619 (Upjohn), a stable prostaglandin endoperoxide analog, further referred to as prostaglandin.

**Methods**

All animals were maintained in accordance with the guidelines of the Committee on Animals of Harvard Medical School and the Guide for Animal Care and Use of Laboratory Animals [DHSS publication (NIH) 85-23, revised 1985].

Successful experiments were conducted in 34 mongrel dogs of either sex (21.3 ± 0.6 kg), anesthetized by iv infusion of a warmed solution of chloralose (150 mg·kg\textsuperscript{-1}) and urethane (1.5 g·kg\textsuperscript{-1}). The lungs were mechanically ventilated with oxygen through a cuffed endotracheal tube by a volume-cycled respirator with a PEEP of 5 cmH\textsubscript{2}O. The chest was opened through a median sternotomy. Initially, 6,000 units of heparin was given iv followed by 1,000 U/h. A circuit was primed with fresh heparinized canine whole blood and total cardiopulmonary bypass instituted. Systemic venous blood drained from the superior vena cava and femoral veins to the pulmonary artery blood reservoir heat exchanger (BOS 10 Shiley) (fig. 1). A pulmonary perfusion cannula was placed in the main pulmonary artery via a right ventriculotomy, and a calibrated pulsatile flow pump was connected to it to perfuse the left lung with warmed venous blood (36.5–38°C) from this reservoir. The left atrium was drained via the appendage to a reservoir adjusted to 10–20 cm below the heart to reduce transmission of changes in left atrial pressure to pulmonary vessels.\textsuperscript{6} The airways and blood vessels of the right lung were excluded by a heavy ligature around the right hilum, and the left lung was mechanically ventilated. The pulmonary blood reservoir supplied a second oxygenator reservoir heat exchanger (BOS 10 Shiley) for separate perfusion of the systemic vessels with warmed oxygenated blood. This was infused via the femoral arteries by a calibrated occlusive roller pump adjusted to hold mean aortic pressure constant to minimize reflexes that could influence the pulmonary vessels. Gas flows to this second oxygenator were adjusted (97% O\textsubscript{2}/3% CO\textsubscript{2} or 95% O\textsubscript{2}/5% CO\textsubscript{2}), and sodium bicarbonate was infused to achieve control blood gas values within a relatively narrow range. Table 1 shows details of values obtained in protocols C1 and C3 and values obtained in corresponding time periods obtained in protocols A and B were similar.

To measure pulmonary artery pressure, a catheter tip pressure transducer (Millar) was positioned through the pulmonary perfusion cannula to protrude about 1.5 cm

**FIG. 1.** The lung preparation in situ. In each experiment, the pulmonary perfusion catheter was introduced through a right ventriculotomy, not shown in this diagram.
### Table 1. Blood Gas Tensions and pH in Pulmonary and Femoral Arterial and Left Atrial Blood in Protocol C Experiments

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Pulmonary Artery</th>
<th>Femoral Artery</th>
<th>Left Atrium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P&lt;sub&gt;02&lt;/sub&gt;</td>
<td>P&lt;sub&gt;CO2&lt;/sub&gt;</td>
<td>pH</td>
</tr>
<tr>
<td>Alveolar hypoxia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-1</td>
<td>46.1 ± 2.8</td>
<td>48.1 ± 2.4</td>
<td>7.31 ± 0.02</td>
</tr>
<tr>
<td>Alv Hx-1</td>
<td>42.8 ± 4.4</td>
<td>48.8 ± 2.7</td>
<td>7.30 ± 0.02</td>
</tr>
<tr>
<td>HXHC 0.25 mM</td>
<td>46.8 ± 3.4</td>
<td>46.1 ± 2.1</td>
<td>7.29 ± 0.02</td>
</tr>
<tr>
<td>Alx Hx-2</td>
<td>41.4 ± 4.2</td>
<td>46.5 ± 2.5</td>
<td>7.30 ± 0.01</td>
</tr>
<tr>
<td>Control-2</td>
<td>46.5 ± 4.5</td>
<td>46.9 ± 3.5</td>
<td>7.31 ± 0.02</td>
</tr>
<tr>
<td>Alv Hx-3</td>
<td>37.1 ± 3.3</td>
<td>46.3 ± 2.6</td>
<td>7.31 ± 0.02</td>
</tr>
<tr>
<td>HXNF</td>
<td>46.9 ± 2.9</td>
<td>43.1 ± 2.8</td>
<td>7.31 ± 0.02</td>
</tr>
</tbody>
</table>

The first column shows the sequence of experimental time periods from above down for each set of experiments.

Alv Hx = alveolar hypoxia; HXHC = hypocalcemia + alveolar hypoxia.

Beyond its tip. To monitor aortic pressure, a 50-cm stiff-walled catheter (flat response up to 30 Hz) was advanced through the right internal mammary artery to the origin of the brachiocephalic trunk. Mean pulmonary artery and mean aortic pressures, determined by electronic integration, were recorded on a direct writing 8-channel oscillograph (Hewlett Packard 8877A®). At the conclusion of the experiment the true zero values were determined by exposing the tip of the catheters in situ to atmospheric pressure.

Because pulmonary artery pressure–flow plots represent a sensitive measure of pulmonary vascular tone, such data were generated where specified in the protocols. Pulmonary blood flow was increased in 250 ml·min⁻¹ increments from 250 ml·min⁻¹ to a maximum of 1250 ml·min⁻¹. At each flow setting, mean pulmonary artery pressure was recorded while mean aortic pressure was constant and when alveolar pressure had fallen to zero due to interruption of mechanical ventilation. Care was taken that at each flow setting no pulmonary artery pressure overshoot was present. All data points forming one plot were recorded within 45 s. Pulmonary blood flow was then returned to its baseline level and pulmonary ventilation resumed. The mean pulmonary artery pressure at constant flow (PAm) and the slope of these plots were derived as described in “Data Analysis.”

Peripheral vascular responses were assessed by the rate of systemic blood flow required to hold mean aortic pressure constant.

Ionized calcium was measured by a calibrated flow-throuth calcium-selective electrode system. The response of this electrode is nearly linear up to [Ca⁺⁺] of 10 mM, i.e., nearly 10 times the physiologic level and more than five times the upper limit of stable hypercalcemia studied in the present experiments. Because analytic results were available within 90 s after withdrawal of the blood sample, it was possible to maintain [Ca⁺⁺] within narrow limits by adjustment of the calcium or citrate infusion rate. This allowed all data points forming a given pulmonary pressure–flow plot to be obtained at the same [Ca⁺⁺].

In all experiments, following an initial 20-min stabilization period with normal [Ca⁺⁺], pulmonary blood flow was set at a rate to yield a mean pulmonary artery pressure between 9 and 15 mmHg; pulmonary blood flow was held constant thereafter, except for temporary interruption during pressure–flow plot data generation. Figure 2 shows the sequence of interventions in each protocol.

### Protocol A: Hypercalcemia

Pulmonary arterial, aortic, and left atrial blood specimens were withdrawn simultaneously before and after calcium bolus administration (protocol A1) or before, during, and after stable hypercalcemia (protocol A2) for [Ca⁺⁺], blood gases and pH, sodium, potassium, and osmolality.

### Protocol A1: Transient Hypercalcemia

With pulmonary blood flow constant, after disconnecting the ventilator tubing, each of five dogs received calcium chloride bolus doses of 5 and 15 mg·kg⁻¹ (encompassing the therapeutic range), injected approximately 20 min apart in random order. Each dose was injected over 2 s into the pulmonary artery perfusion can-
nula, and pulmonary artery pressure was recorded. Ventilation was resumed after each sodium dose. Normocalemia, restored by adding appropriate aliquots of a buffered trisodium citrate solution to the pulmonary blood reservoir, was documented prior to the second injection.

**PROTOCOL A2: STABLE HYPERCALCemia**

We instituted stable hypercalcemia ([Ca^{++}] = 1.88 ± 0.03 mM) in another group of six dogs to obtain pulmonary pressure–flow plot data during initial normocalcemia, stable hypercalcemia, and a second normocalcemic period, each 30 min in duration (fig. 2). Stable hypercalcemia was created by adding calcium chloride to the pulmonary artery blood reservoir followed by a slow infusion at a rate adjusted as necessary on the basis of frequent [Ca^{++}] measurements. Normocalcemia was restored as in protocol A1.

**PROTOCOLS B AND C: HYPOCALCemia**

Stable hypocalcemia was induced by infusing buffered trisodium citrate solution into the pulmonary artery reservoir at rates sufficient to yield stable [Ca^{++}] at the desired level of hypocalcemia, adjusted on the basis of and documented by repeated [Ca^{++}] measurements. In four hypocalcemia protocols, pulmonary vasomotor tone was first increased, either by alveolar hypoxia (protocols B, C1, and C2) or by infusion of prostaglandin (protocol C3) (fig. 2). These vasoconstrictor interventions were necessary because at baseline pulmonary vasomotor tone is low and possible effects of any vasodilator may not be seen. Alveolar hypoxia was produced and maintained by ventilating the lung with 100% nitrogen. Mechanical dead space was adjusted and sodium bicarbonate added to yield PaO₂ and pH values in left atrial blood within relatively narrow limits; values for protocols C1 and C3 are shown in table 1. Values obtained in protocol B were similar to those in the corresponding time periods of protocol C1. To prevent fading of the hypoxic pulmonarypressor response, whether alveolar hypoxia was induced or not, in all dogs of the hypocalcemia protocols, sodium ibuprofenate 2 mg/kg (Upjohn) was injected into the pulmonary blood reservoir at the initiation of cardiac bypass, followed by 2 mg · kg⁻¹ · h⁻¹.

We first documented that the lung was capable of developing a reversible pulmonarypressor response to alveolar hypoxia or prostaglandin infusion. In protocols C1 and C2, alveolar hypoxia, at constant pulmonary blood flow, increased mean pulmonary artery pressure to approximately 60% above control followed by a return to control by ventilation with oxygen. In protocol C3 the prostaglandin was infused to produce an increase in mean pulmonary artery pressure similar to that with alveolar hypoxia, which was followed by a return of mean pulmonary artery pressure to control when the prostaglandin infusion was discontinued. These initial periods were not further analyzed and are not included in figure 2. Next, pulmonary pressure–flow data and systemic hemodynamic data were collected at the end of the experimental periods, each 30-min in duration in the sequences shown in figure 2. Pulmonary arterial, left atrial, and aortic blood specimens were withdrawn at the end of each experimental period.

**PROTOCOL B: HYPOCALCemia**

To determine what level of hypocalcemia is required to observe any pulmonary artery pressure changes while alveolar hypoxia was maintained, sequential stable [Ca^{++}] levels of 0.8, 0.5, and 0.26 mM were instituted (fig. 2). At each hypocalcemia level, pulmonary pressure–flow data were generated.
PULMONARY VASCULAR RESPONSES TO HYPERCALCEMIA AND HYPOCALCEMIA

PROTOCOL C: HYPOCALCEMIA VS. NIFEDIPINE

In six dogs (protocol C1), the effects of severe hypocalcemia ([Ca\(^{++}\)] = 0.27 ± 0.01 mM) were compared with those of a large dose of nifedipine (10 \(\mu\)g kg\(^{-1}\) as a bolus followed by continuous infusion at a rate of 40 \(\mu\)g kg\(^{-1}\) h\(^{-1}\)) using pulmonary pressure–flow plots. The nifedipine solution was prepared as previously described; all syringes and infusion tubing were shielded from light. In these dogs hypocalcemia was always tested before nifedipine (fig. 2). To test whether time and/or the preceding hypocalcemia influenced the pulmonary vascular response to nifedipine, in an additional four dogs (protocol C2) nifedipine was studied both before and after hypocalcemia (fig. 2); thus, the effects of nifedipine as the first intervention were compared with those of nifedipine as the last intervention in the same animal.

In eight dogs (protocol C3), prostaglandin was infused (0.045 ± 0.04 \(\mu\)g kg\(^{-1}\) min\(^{-1}\)) during pulmonary ventilation with 100% oxygen; mechanical dead space was adjusted to achieve blood gas and pH values shown in table 1. Next, while prostaglandin infusion was maintained, hypocalcemia ([Ca\(^{++}\)] = 0.23 ± 0.01 mM) and nifedipine (bolus of 10 \(\mu\)g kg\(^{-1}\) min\(^{-1}\) followed by 40 \(\mu\)g kg\(^{-1}\) h\(^{-1}\)) were studied with pulmonary pressure–flow plots (fig. 2).

Mean aortic pressure was constant at 75 mmHg throughout most experiments, but in several experiments we could not achieve that level of aortic pressure. Therefore, we studied the effect of mean aortic pressure on pulmonary pressure–flow plots (n = 5). These control experiments were performed in one animal each of protocols B and C1, and in three animals of protocol C3, prior to the start of the respective protocols. After a stabilization period of 20 min, pulmonary pressure–flow plots were generated at different mean aortic pressures ranging from 40 to 110 mmHg. Each plot was obtained after the selected mean aortic pressure had been maintained for 10 min.

To exclude an effect of the nifedipine solvent, a solution containing all components except the nifedipine itself was tested in four animals of protocol C1 and in two animals of protocol C3, immediately prior to testing nifedipine.

DATA ANALYSIS

A pulmonary vasoactive effect was considered to be present when a change occurred in mean pulmonary artery pressure at zero airway pressure, constant pulmonary blood flow, and pulmonary outflow pressure. In protocol A1, pulmonary pressure–flow plots were constructed by plotting mean pulmonary artery pressure against the corresponding blood flow. A left and upward shift of this plot indicates greater pulmonary artery pressure at any flow, i.e., pulmonary vasoconstriction, whereas a right and downward shift indicates a lesser pulmonary artery pressure at any flow, i.e., vasodilation. We evaluated these plots by comparing their slopes and PAm, measured at the highest common pulmonary blood flow, which was constant for each experiment. Peripheral vascular responses were assessed by the rate of systemic blood flow to hold mean aortic pressure constant; for example, at constant pressure, greater flow indicates vasodilation.

To have greater power for comparisons of the slopes associated with the interventions within a protocol, we analyzed the data from each protocol according to a multiple regression model. This model had pulmonary artery pressure as the dependent variable and the number of explanatory variables was twice the number of interventions. Specifically, in addition to the overall constant (which served as the intercept for the first intervention), the explanatory variables consisted of a flow variable for each intervention (giving the values of blood flow for that intervention and taking the value zero for all other interventions) and an indicator variable for each intervention after the first (taking the value 1 for that intervention and 0 elsewhere, and thus corresponding to the difference in intercept between that intervention and the first intervention). Although this multiple regression model yields the same fitted line segments for the interventions as one would get by applying simple linear regressions to each intervention separately, it has the advantage of combining the deviations from all these fitted lines into a single estimate of the residual variance (\(\sigma^2\)). This estimate has more degrees of freedom than would be available from fitting a regression line to any single intervention or from fitting a simple multiple regression model to any pair of interventions. As a result, the \(t\) tests for comparing slopes, which all use this estimate of \(\sigma^2\) in their denominators, have greater power. The appendix provides additional details of the multiple regression model.

In addition, because the observations within each study period of a protocol were obtained successively over a brief period of time, they may be correlated. The multiple regression framework facilitates assessment of this serial correlation and, if necessary, adjustment for this departure from the usual assumptions according to a modification of the Cochrane-Orcutt procedure. In the present data, the actual estimates of serial correlation were generally close to zero; thus, no adjustment was necessary. From the results of the multiple regression, we calculated delta slope values and their standard errors for each intervention. For example, in protocol A2 the delta slope value was determined for each experiment as the difference between the slope during stable hypercalcemia and the slope during the immediately preceding control period. In protocol C one set of delta slope values came
from the slope obtained for alveolar hypoxia (or prostaglandin) and the slope for the immediately preceding control period. Additional sets of delta slope values came from hypocalcemia (or nifedipine) and the respective immediately preceding alveolar hypoxia (or prostaglandin) periods. Data obtained in protocol B were analyzed in a similar manner. T statistics (based on the appropriate standard errors) were used to compare alveolar hypoxia or prostaglandin periods with their control periods and to compare the effects of hypocalcemia to the effects of nifedipine. Group means of PAm as listed in table 2 were compared as sample means.

All comparisons made allowance for multiple testing. Significance of individual comparisons was judged according to Bonferroni t tests using a simultaneous level of 0.05, i.e., 0.05 was divided by the number of comparisons to obtain the individual level that an individual P value must satisfy to achieve significance. For example, with four comparisons at a simultaneous 0.05 level of significance, one compares each of the four P values to 0.05/4 or 0.0125. Where more than one comparison was made, statements in the text of statistical significance for individual comparisons report the resulting exact level, as in "P = 0.008," and also the simultaneous level and the number of comparisons considered, as in "P < 0.05/4." The figures and tables provide the individual P values.

Results

HYPERCALCEMIA

Transient hypercalcemia. Mean pulmonary artery pressure remained unchanged after calcium chloride doses of 5 and 15 mg/kg.

Stable hypercalcemia. In all experiments, pulmonary pressure–flow plots obtained during initial and second control periods and stable hypercalcemia were virtually superimposable (fig. 3). Thus, the slopes and PAm during control and stable hypercalcemia were not significantly different (table 2). Of 18 plots, correlation coefficients were greater than 0.95 in 9 and less than 0.90 in 3. There was a decrease in systemic blood flow from 1.45 ± 0.21 to 1.22 ± 0.17 l/min (P = 0.008), which returned to near-control (1.59 ± 0.32 l/min) when [Ca++] returned to control.

HYPOCALCEMIA

In all experiments, alveolar hypoxia and prostaglandin infusion displaced the pressure–flow plot upward and to the left (fig. 4), as expected. Thus, the slope and PAm increased (table 2). With repeated alveolar hypoxic challenges these changes became greater, in accord with previous data.21,25 There were decreases in systemic blood flow during alveolar hypoxia (0.88 ± 0.06 vs. 0.73 ± 0.03 l/min, P = 0.023) and during prostaglandin infusion (0.97 ± 0.09 vs. 0.73 ± 0.05 l/min, P = 0.002).

Different degrees of hypocalcemia. Moderate hypocalcemia (0.8–0.5 mM) did not significantly alter the slope of the pressure–flow plots or PAm (P > 0.05/4). In contrast, severe hypocalcemia ([Ca++] = 0.26 ± 0.01 mM) decreased the slope (P = 0.0001 < 0.05/4) and PAm (P = 0.002 < 0.05/4, fig. 5).

Severe hypocalcemia vs. nifedipine. Pulmonary pressure–flow plots obtained in representative experiments are shown in figure 4. Alveolar hypoxia increased the slope and shifted the plot upward and to the left, as expected. Group data for alveolar hypoxia are shown in table 2. Values for slope during alveolar hypoxia were greater than the corresponding control periods (P = 0.0001 < 0.05/4). Corresponding values of PAm were also greater during alveolar hypoxia (P = 0.0001 < 0.05/4).
Nifedipine decreased the slope and shifted the plot downward and to the right (fig. 4). Although in protocol C2 the average decrease in PAm (delta PAm) during the first nifedipine application was similar to that during the second (−7.9 vs. −9.3 mmHg, $P = 0.6$), the decrease in slope (delta slope) of the pressure-flow plot during the first nifedipine application was less than that during the second (−12.1 vs. −28.7 mmHg $\cdot$ min$^{-1}$, $P = 0.005$). Therefore, the experimental sequence or time may have influenced the results obtained with nifedipine. Results obtained in protocol C2 were pooled with results of the corresponding time periods in protocol C1, i.e., data from the hypocalcemia periods in C1 and C2 were pooled, and

![Graph](image)

**Fig. 4. A and B.** Pulmonary artery pressure-flow plots obtained with hypocalcemia (A) and nifedipine (B) during alveolar hypoxia. B. The last plot obtained in panel A and data obtained later. The sequence of interventions is indicated from above down in the key in each panel. During normocalcemia ([Ca$^{2+}$] = 1.06 ± 0.02 mM) alveolar hypoxia increased the slope and shifted the plot upward and to the right. Severe hypocalcemia ([Ca$^{2+}$] = 0.27 mM) and nifedipine (bolus of 10 $\mu$g $\cdot$ kg$^{-1}$ followed by 40 $\mu$g $\cdot$ kg$^{-1}$$\cdot$min$^{-1}$) decreased the slope and shifted the plot downward and to the right. Calcium chloride infused to restore normocalcemia after hypocalcemia also restored the slope and position of the plot first produced with alveolar hypoxia. Withdrawal of the alveolar hypoxic stimulus then shifted the plot to its control position. C and D. Pulmonary artery pressure versus flow plots obtained with hypocalcemia (C) and nifedipine (bolus of 10 $\mu$g $\cdot$ kg$^{-1}$ followed by 40 $\mu$g $\cdot$ kg$^{-1}$$\cdot$min$^{-1}$) during prostaglandin infusion (D). The last plot obtained in panel C and data obtained later. The sequence of interventions is indicated from above down in the key in each panel. During normocalcemia ([Ca$^{2+}$] = 1.10 ± 0.02 mM) the prostaglandin increased the slope and shifted the plot upward and to the left, and although the pattern of response to hypocalcemia and nifedipine was similar to that seen during alveolar hypoxia, the effects of nifedipine were less. Calcium chloride infused to restore normocalcemia after hypocalcemia also restored the slope and position of the plot first produced with prostaglandin infusion.

![Graph](image)

**Fig. 5.** Pulmonary artery pressure-flow plots during stable hypercalcemia ([Ca$^{2+}$] = 1.88 ± 0.05 mM) illustrated in three experiments. The plots obtained with hypercalcemia were virtually superimposable on those obtained during normocalcemia.

data from the nifedipine period in C1 were pooled with data from the second nifedipine period in C2. Hypocalcemia also decreased the slope and shifted the plot downward and to the right. Delta PAm during hypocalcemia was similar to that during nifedipine ($P = 0.7 > 0.05/4$), but delta slope was smaller with hypocalcemia than with nifedipine ($P = 0.002 < 0.05/4$, fig. 6). Of 60 plots, correlation coefficients were greater than 0.95 in 44 and less than 0.9 in 3. The increase in systemic flow with hypocalcemia was greater than that with nifedipine ($P = 0.002$, fig. 7, upper panel).

**Prostaglandin.** Pressure-flow plots obtained in representative experiments are shown in figure 4. Prostaglandin...
**Fig. 5.** Pulmonary vascular responses to different levels of hypocalcemia in the presence of alveolar hypoxia. Although moderate degrees of hypocalcemia ([Ca++]) 0.8 and 0.5 mM did not influence mean pulmonary artery (PAm) pressure at the highest common flow or slope, severe hypocalcemia ([Ca++] = 0.26 ± 0.01 mM) reduced both. The individual significance levels (compared with respective alveolar hypoxic periods) are shown. These hypocalcemia data are not included in the results shown in figure 6.

**Fig. 6.** Comparative effects of severe hypocalcemia and nifedipine in the presence of alveolar hypoxia and prostaglandin, expressed by delta PAm and delta slope, alveolar hypoxia versus prostaglandin. Nifedipine was given as a bolus (10 µg/kg) followed by continuous infusion (40 µg·kg⁻¹·h⁻¹). The individual significance levels shown refer to comparisons between delta values. For the same changes produced with hypocalcemia (hypoxia vs. prostaglandin), the reduction in slope of PAm with nifedipine was much greater during alveolar hypoxia than during prostaglandin infusion.

**Fig. 7.** Comparative systemic vascular effects of hypocalcemia and nifedipine in the presence of alveolar hypoxia and prostaglandin infusion. During alveolar hypoxia, the increase in systemic flow with hypocalcemia was greater than that with nifedipine. Significance levels shown refer to comparison between delta values.

Infusion increased the slope and shifted the plot upward and to the left, as expected. With repeated alveolar hypoxic challenges these changes became greater. Group data for prostaglandin are shown in table 2. PAm and slope were greater during prostaglandin than corresponding control (both \( P = 0.0001 < 0.05/4 \)). Hypocalcemia decreased the slope and shifted the plot downward and to the right. However, there was only a slight change in slope or position with nifedipine (fig. 4). A comparison of hypocalcemia with nifedipine indicates that hypocalcemia had the greater effect on PAm (\( P = 0.011 < 0.05/4 \)) and slope (\( P = 0.002 < 0.05/4 \)) (fig. 6). Of 48 plots, correlation coefficients were greater than 0.95 in 40 and smaller
than 0.9 in 4. The increase in systemic flow with hypercalcemia was not significantly different from that with nifedipine ($P = 0.19$, fig. 7, lower panel).

Effects of hypocalemia and nifedipine during alveolar hypoxia were compared with their effects during prostaglandin (fig. 6). Hypocalcemia reversed the vasoconstriction produced by alveolar hypoxia and prostaglandin to the same extent (fig. 6), as shown by similar delta slope ($P = 0.9 > 0.05/4$) and delta PAmp ($P = 0.85 > 0.05/4$). Although nifedipine had an equal (PAm) or greater (slope) effect than hypocalcemia during hypoxia, nifedipine had a substantially smaller effect than hypocalcemia during prostaglandin infusion (delta slope with nifedipine during alveolar hypoxia vs. during prostaglandin, $P = 0.0001 < 0.05/4$; delta PAmp with nifedipine during alveolar hypoxia vs. during prostaglandin, $P = 0.011 < 0.05/4$).

Control experiments. Pressure–flow plots obtained at different mean aortic pressures or with nifedipine blank solution were each nearly superimposable.

Biochemical values. In each dog hematocrit varied not more than 8 vol%, plasma osmolality not more than 20 mosm/kg, sodium not more than 15 mM, and potassium not more than 0.7 mM from control.

Discussion

Although in normal humans [Ca$^{++}$] is held within narrow limits (1.10 mM ± 5%),$^{36}$ an approximate fourfold range of [Ca$^{++}$] values may be seen in patients. With hypocalcemia, values as low as 0.5 mM and with hypercalcemia up to 1.9 mM$^{26,27}$ have been observed. The most important finding of this study is that the pulmonary vessels are unresponsive to [Ca$^{++}$] changes within this range of values. Furthermore, a reduction of [Ca$^{++}$] to 25% of control is required to produce a degree of pulmonary vasodilation that is comparable with an established dose of nifedipine.

Animal Preparation

Data were collected at zero airway pressure in the whole-blood perfused canine lung in situ. Because a simple calculation of pulmonary vascular resistance can be misleading in the assessment of true flow resistance of the pulmonary vessels,$^{38}$ we controlled pulmonary blood flow and pulmonary outflow pressure as described previously.$^6$ We also controlled aortic pressure to minimize reflex-induced changes in the pulmonary vessels.$^{22}$ With these experimental conditions, any vasoconstrictor action of a calcium bolus infusion would be shown by increased mean pulmonary artery pressure.$^{22}$

We also studied the pulmonary vessels by pulmonary artery pressure–flow plots during stable hypercalcemia and hypocalemia. These plots are rectilinear (fig. 4), and their slopes are sensitive measures of resistance.$^{28}$

We evaluated the pulmonary artery pressure–flow plot using PAm. The slopes observed during control periods and alveolar hypoxia were similar to those reported by others.$^{19,29}$ In each experiment the highest pulmonary blood flow rate was selected to ensure that mean pulmonary artery pressure during vasoconstrictor interventions would not exceed 35–40 mmHg because in preliminary experiments in which these values had been exceeded, the pulmonary vessels became rapidly unstable. This instability was shown by rapid spontaneous increases in PA pressure to above 60 mmHg at flows below 0.8 1·min$^{-1}$. Data obtained in protocol C2 show that time alone is a possible factor in the smaller vasodilator effect of hypocalcemia, as measured by delta slope, than that of nifedipine. This makes the smaller change with nifedipine compared with hypocalcemia during prostaglandin infusion (protocol C3) all the more striking as discussed below. In our model, pulmonary artery pressure was recorded in the presence of anesthesia with chloralose and urethane, after surgical dissection, and with the use of controlled circulation. Furthermore, possible interspecies differences in pulmonary responses are recognized. Thus, there are some limitations to the immediate clinical application of these experimental data.

Bolus Calcium Infusion

We tested the pulmonary pressor effects of low and high calcium bolus doses, encompassing the therapeutic range. Because we injected calcium chloride directly into the pulmonary artery, the initial volume of distribution was less than when injected via clinically employed routes of administration. Therefore, it is likely that peak [Ca$^{++}$] levels in blood perfusing the lung were greater than those obtained clinically. However, mean pulmonary artery pressure remained unchanged with both calcium doses, indicating that calcium in doses used clinically does not alter pulmonary vascular resistance. This lack of change cannot be attributed to a general unresponsiveness of our lung preparation because pulmonary vasoconstriction was observed as expected with the application of alveolar hypoxia or prostaglandin.

Stable Hypercalcemia and Hypocalcemia

Pulmonary artery pressure–flow plots obtained during hypercalcemia and normocalcemia were nearly superimposable (fig. 3); thus, their slopes and PAm values were similar (table 2). These experiments confirm data obtained with calcium bolus infusion, showing that the pulmonary vessels are insensitive to [Ca$^{++}$] changes in the clinical hypercalcemia range. These findings may have important clinical implications, particularly for patients undergoing cardiac surgery. The data suggest that calcium can be safely given via a right-sided catheter and does not need...
to be administered, like other vasoressor drugs, via a left atrial catheter, to achieve a cardiac effect without undesirable pulmonary vasoconstriction. However, the general limitations of extrapolating experimental data to the clinical situation must be recognized. Furthermore, we cannot exclude that clinical levels of hypercalcemia may have pulmonary vasoconstrictor action when vasoconstriction by other causes is already present.

We tested the vasodilator action of hypercalcemia by gradually lowering $[\text{Ca}^{++}]$ and documenting the ability of each $[\text{Ca}^{++}]$ level to decrease PAm or the slope of the pulmonary artery pressure–flow plot, which had been first increased by alveolar hypoxia. The data show little change in slope of these pressure–flow plots or PAm at [Ca$^{++}$] values down to near 50% of normal, indicating that the pulmonary vessels are minimally responsive to clinically encountered levels of hypercalcemia. These observations are of interest in patients during anesthesia and in the critical care setting because vasodilators antagonize alveolar hypoxic vasoconstriction and thereby may increase pulmonary shunting and arterial desaturation. This problem is unlikely with the clinically encountered degrees of hypercalcemia.

Severe hypercalcemia ([Ca$^{++}$] near 25% of normal) was required to produce pulmonary vasodilatation, as shown by a significant decrease in the slope of the pressure–flow plot and PAm. It is emphasized, however, that this severe degree of hypercalcemia is not useful clinically as a pulmonary vasodilator because cardiac function is not supported at such a low [Ca$^{++}$] level. These findings are consistent with data obtained in isolated perfused rabbit pulmonary artery in situ showing vasodilation with a calcium-free perfusate.

**Severe Hypocalcemia vs. Nifedipine**

The data demonstrate that nifedipine was associated with a decrease in slope of the pulmonary artery pressure–flow plot and PAm, as expected. With hypocalcemia, delta slope and PAm values were similar during alveolar hypoxia and prostaglandin infusion, but the nifedipine effects were much smaller during prostaglandin infusion than during alveolar hypoxia (fig. 6). It is nevertheless possible that prostaglandin-induced pulmonary vasoconstriction might have been completely reversed by a higher nifedipine dose. However, during prostaglandin infusion we tested the dose of nifedipine that produced an average delta PAm similar to that produced by severe hypocalcemia during alveolar hypoxia; both hypocalcemia and nifedipine almost completely reversed hypoxic pulmonary vasoconstriction.

Although both hypocalcemia and nifedipine reduce calcium influx, hypocalcemia did not produce the differential effects seen with nifedipine. This contrast between hypocalcemia and nifedipine was unexpected. The data do not elucidate the mechanism of pulmonary vasoconstriction nor allow conclusions on molecular mechanisms of smooth muscle contraction. However, one possible explanation for these different pulmonary artery responses to severe hypocalcemia and nifedipine could be the two different mechanisms proposed to account for the way the calcium ion acts as a coupler of excitation to contraction.

Electromechanical coupling is the process wherein the calcium ions act as transducers of electrical signals across the smooth muscle membrane, as they carry slow inward current in smooth muscle. Membrane depolarization is associated with calcium influx via voltage-operated channels, which have a gating mechanism sensitive to changes in membrane potential. Changes in calcium conductance in vascular smooth muscle cells occur during alveolar hypoxia, although the exact mechanism remains unknown. For example, electrical and mechanical responses recorded in small pulmonary arteries of the cat as $P_{O_2}$ was lowered from 300 to below 50 mmHg showed that depolarization and active force developed when low $P_{O_2}$ values were reached. The amplitude of hypoxic contraction decreased when exposure to low $P_{O_2}$ was combined with decreased perfuse calcium concentration and was abolished with verapamil. Thus, hypoxia-related, calcium-dependent membrane depolarization occurred with action potential generation when $P_{O_2}$ was low. The evidence for the involvement of voltage-operated channels in this mechanism comes from experiments with nifedipine, which is particularly effective in reducing calcium flux via the voltage-operated channels as shown by its ability to antagonize the vasoconstrictor effects of interventions selectively acting on the voltage-operated channels, including the calcium promotor BAY K8644 and potassium chloride. Calcium blockers do not appear to affect the process of sodium–calcium exchange or intracellular calcium mobilization as shown in skinned muscle preparations.

Pharmacomechanical coupling is a mechanism wherein calcium ions act as transducers of chemical signals; calcium influx occurs via receptor-operated calcium channels and smooth muscle contraction occurs independent of membrane depolarization. Experiments with the thromboxane receptor blockers SQ25948 and BM13-177 support the hypothesis that some prostaglandin compounds act by direct stimulation of thromboxane receptors both in the airways and in the pulmonary vessels. The role of the calcium ion in the response to thromboxane receptor stimulation is suggested by $^{45}$Ca studies in the isolated rabbit aorta. In these studies, the prostaglandin analog U44069 produced an intracellular gain in calcium, which was less after reduction of extracellular calcium. In other experiments thromboxane-induced va-
soconstriction in the isolated rabbit pulmonary artery was inhibited by a calcium-free perfusate. Another study showed that whereas nifedipine weakly antagonized vasocostriction produced with prostaglandin F2α, it nearly abolished vasoconstriction by hypoxia. Our nifedipine data are consistent with these findings.

With preexisting hypocalcemia in the presence of alveolar hypoxia or prostaglandin, calcium chloride infusion had pulmonary vasopressor action, as shown by the increase in slopes and PAm (fig. 4). These observations make it unlikely that pulmonary vasodilation observed with hypocalcemia were due to effects of citrate on other ionic species or to effects of citrate per se.

PULMONARY VS. PERIPHERAL VASCULAR RESPONSES

The data show that stable hypercalcemia increased systemic vascular resistance as shown by a reversible decrease in the systemic blood flow to hold mean aortic pressure constant. Conversely, with severe stable hypocalcemia, systemic blood flow increased. These findings confirm previous data on peripheral vascular effects of hypercalcemia obtained in the intact animal. The clinical importance of these findings is that the peripheral blood vessels contribute to the hemodynamic consequences of hypercalcemia. Considered together with the pulmonary vascular effects, the data show disparate pulmonary and systemic vascular responses to changes in \([\text{Ca}^{++}]\). This is consistent with known disparate actions of other vasopressor interventions.

In conclusion, this study shows an insensitivity of the pulmonary vessels to \([\text{Ca}^{++}]\) changes within the clinical hypercalcemia and hypocalcemia ranges. A reduction of \([\text{Ca}^{++}]\) to 25% of normal is required to produce pulmonary vasodilation. However, such severe hypocalcemia is outside the clinical range. Calcium infusion then reversed the pulmonary vasodilation produced with this severe degree of hypocalcemia. Comparative data show that whereas the pulmonary vasodilator effects of hypocalcemia \(([\text{Ca}^{++}] \text{reduced to 25% of control})\) are independent of the intervention first inducing pulmonary vasosconstriction, those of a high dose nifedipine are much more pronounced during alveolar hypoxia than during prostaglandin infusion.

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References


Appendix

In one common notation the multiple regression model relating the dependent variable Y to the explanatory variables X_1, X_2, \cdots, X_p is

\[ Y = \beta_1 X_1 + \beta_2 X_2 + \cdots + \beta_p X_p + \epsilon. \]

The parameters \( \beta_1, \beta_2, \cdots, \beta_p \) are the regression coefficients, and \( \epsilon \) is a random fluctuation, assumed to come from a distribution with mean 0 and variance \( \sigma^2 \). Also, the fluctuations associated with different observations on \( Y \) are assumed to be uncorrelated. Usually \( X_1 \) has the constant value of 1, so that \( \beta_1 \) is the intercept.

When one fits the above model to \( n \) observations on \( Y \) and \( X_1, X_2, \cdots, X_p \), the result for observation \( i \) can be written

\[ Y_i = b_1 X_{1i} + b_2 X_{2i} + \cdots + b_p X_{pi} + \epsilon_i. \]

Each \( b \) is an estimate of \( \beta \) and \( \epsilon \) (the deviation of \( Y_i \) from the fitted regression equation) is a residual. The customary estimate of \( \sigma^2 \) is

\[ s^2 = (\epsilon_1^2 + \epsilon_2^2 + \cdots + \epsilon_n^2)/(n-p). \]

For protocol C1, for example, \( p = 14 \), \( Y \) is pulmonary artery pressure, and the explanatory variables are as follows:

\[ X_1 = \text{the constant (1 for each observation)}; \]
\[ X_2 = \text{blood flow during the initial control period (and 0 during the other periods)}; \]
\[ X_3 = \text{the indicator for the first period of alveolar hypoxia (1 for each observation during the first alveolar hypoxia period and 0 otherwise)}; \]
\[ X_4 = \text{blood flow during the first period of alveolar hypoxia (and 0 during the other periods)}; \]
\[ X_5 = \text{the indicator for alveolar hypoxia in combination with hypocalcemia}; \]
\[ X_6 = \text{blood flow during alveolar hypoxia in combination with hypocalcemia}; \]
\[ X_7 = \text{the indicator for the second period of alveolar hypoxia}; \]
\[ X_8 = \text{blood flow during the second period of alveolar hypoxia}; \]
\[ X_9 = \text{the indicator for alveolar hypoxia in combination with nifedipine}; \]
\[ X_{14} = \text{blood flow during alveolar hypoxia in combination with nifedipine}. \]

The regression coefficients have the following interpretations:

\[ \beta_1 \] is the intercept for the initial control period;
\[ \beta_2 \] is the slope of pulmonary artery pressure against flow for the initial control period;
\[ \beta_3 \] is the incremental intercept for the first period of alveolar hypoxia, relative to the intercept for the initial control period;
\[ \beta_4 \] is the slope of pulmonary pressure against flow for the first period of alveolar hypoxia;
\[ \beta_{14} \] is the incremental intercept for alveolar hypoxia in combination with nifedipine; and
\[ \beta_{15} \] is the slope of pulmonary artery pressure against flow for alveolar hypoxia in combination with nifedipine.

In this model the estimates of the slopes (\( b_2, b_4, \cdots, b_{14} \)) are uncorrelated. Thus, for comparisons between slopes, the standard error of a difference such as \( b_4 \) minus \( b_3 \) can be calculated from the standard errors of the two slopes (\( b_4 \) and \( b_3 \)).