Effect of changes in ionized calcium concentration in arterial blood and metabolic acidosis on the arterial partial pressure of oxygen in dogs

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Objective—To evaluate the effects of metabolic acidosis and changes in ionized calcium (Ca^{2+}) concentration on Pa_{O_2} in dogs.

Animals—33 anesthetized dogs receiving assisted ventilation.

Procedure—Normal acid-base status was maintained in 8 dogs (group I), and metabolic acidosis was induced in 25 dogs. For 60 minutes, normocalcemia was maintained in group I and 10 other dogs (group II), and 10 dogs were allowed to become hypercalcemic (group III); hypocalcemia was then induced in groups I and II. Groups II and IV (5 dogs) were treated identically except that, at 90 minutes, the latter underwent parathyroidectomy. At intervals, variables including Pa_{O_2}, Ca^{2+} concentration, arterial blood pH (pHa), and systolic blood pressure were assessed.

Results—In group II, Pa_{O_2} increased from baseline value (96 ± 2 mm Hg) within 10 minutes (pHa, 7.33 ± 0.001); at 60 minutes (pHa, 7.21 ± 0.02), Pa_{O_2} was 108 ± 2 mm Hg. For the same pHa decrease, the Pa_{O_2} increase was less in group III. In group I, hypocalcemia caused Pa_{O_2} to progressively increase (from 95 ± 2 mm Hg to 104 ± 3 mm Hg), which correlated (r = –0.66) significantly with a decrease in systolic blood pressure (from 156 ± 9 mm Hg to 118 ± 10 mm Hg). Parathyroidectomy did not alter Pa_{O_2} values.

Conclusions and Clinical Relevance—Induction of hypocalcemia and metabolic acidosis each increased Pa_{O_2} in anesthetized dogs, whereas acidosis-induced hypercalcemia attenuated that increase. In anesthetized dogs, development of metabolic acidosis or hypocalcemia is likely to affect ventilatory control. (*Am J Vet Res* 2006;67:801–808)

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Abbreviations

PTH	Parathyroid hormone
Ca^{2+}	Ionized calcium
pHa	Arterial blood pH

Acidosis and hypocalcemia commonly develop in many critically ill dogs[1–3] and also in some dogs undergoing general anesthesia.[4–6] In these dogs, it is important to tightly control Pa_{O_2} to ensure normal blood oxygenation. In studies[7,8] in dogs, our research group determined that metabolic acidosis directly stimulates PTH secretion, that the acidosis-induced increase in arterial Ca^{2+} concentration reduces the magnitude of PTH stimulation during metabolic acidosis, and that metabolic acidosis enhances the stimulation of PTH secretion during the induction of hypocalcemia. In those studies, arterial blood gases were measured, and as a result, we became aware that both metabolic acidosis and changes in arterial Ca^{2+} concentration affected Pa_{O_2} values.

Metabolic acidosis is known to increase Pa_{O_2} values in dogs. The prevailing opinion is that the increase in Pa_{O_2} results from a reduction in the alveolar-to-arterial O_2 difference caused by a shift in the oxygen-hemoglobin dissociation curve (Bohr effect).[9,10] In an earlier study,[11] it was suggested that the metabolic acidosis-induced increase in Pa_{O_2} might result from a more homogeneous distribution between ventilation and perfusion in the lungs. However, none of those studies evaluated the effect of the increase in arterial Ca^{2+} concentration that occurs during metabolic acidosis. The purpose of the study reported here was to evaluate the effects of metabolic acidosis and changes in arterial blood Ca^{2+} concentration on Pa_{O_2} values in anesthetized dogs.

Materials and Methods

Healthy mixed-breed dogs (18 males and 13 females) that were 2 to 5 years old were used in the study. The mean ± SE weight of the dogs was 26 ± 2 kg, and each was in good body condition (body score, 3/4). All animals received humane care in compliance with the Principles of Laboratory Animal Care, formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences.[12] Experimental protocols were reviewed and approved by the Ethics Committee for Animal Research of the Universidad de Cordoba.

After withholding of food for 12 hours, dogs were premedicated with ketamine[a] (7.5 mg/kg, IM), fentanyl[b] (75

µg/kg, IM), and droperidol[c] (375 µg/kg, IM). In each dog, the left femoral artery, the right jugular vein, and both cephalic veins were cannulated. A bolus of sodium thiopental[d] (12.5 mg/kg) was then given IV to induce anesthesia and facilitate tracheal intubation. After placement of the endotracheal tube, ventilation was controlled with a variable rate respirator.[e] The oxygen concentration (F_{IO_2}) in the inspired gas mixture was set at 27% to maintain Pa_{O_2} at 95 to 105 mm Hg. Ventilation was set at 12 breaths/min with a tidal volume of 15 mL/kg to achieve and maintain Pa_{CO_2} of 30 to 40 mm Hg. Anesthesia was maintained during the experiment via intermittent IV administration of fentanyl[b] (2 µg/kg), midazolam[f] (0.25 mg/kg), and pancuronium bromide[g] (0.1 mg/kg). The arterial catheter was used for blood sampling and blood pressure monitoring.[h] The cephalic vein catheters were used to infuse HCl,[i] EDTA,[j] and 5% dextrose-saline (0.45% NaCl) solution. The jugular vein catheter was used to infuse anesthetic agents and, when required, magnesium sulfate[k] to prevent development of hypomagnesemia during the EDTA infusion.

Dogs were randomly allocated to 1 of 4 groups. The experimental protocols for each group were as follows: normal acid-base status maintained and hypocalcemia induced after a 60-minute period during which dogs remained normocalcemic (group I), metabolic acidosis induced and hypocalcemia induced after a 60-minute period during which dogs remained normocalcemic (group II), metabolic acidosis induced and dogs allowed to become hypercalcemic (group III), and treatment identical to that of group II with parathyroidectomy performed during the period of hypocalcemia (group IV). In all groups, baseline blood samples were obtained at 15, 10, and 5 minutes prior to the start of the experiment and immediately before the start of the experiment (time 0). The duration of the experimental period was 120 minutes in groups I and II and 60 minutes in group III; in group IV, the experimental design was the same as in group II except that parathyroidectomy was performed at 90 minutes. Arterial blood samples were collected into tubes containing heparin every 10 minutes during the experimental period in groups I, II, and III; in group IV, samples were collected every 10 minutes during the first 60 minutes and every 5 minutes from 60 to 120 minutes, with an additional sample collected 2.5 minutes after parathyroidectomy.

Experimental protocol for group I—For the first 60 minutes, the 8 dogs allocated to group I received 5% dextrose-saline (0.45% NaCl) solution IV to match volume load in the other groups. After 60 minutes, hypocalcemia was induced via administration of an EDTA solution (280 mg of EDTA/kg of body weight of EDTA in 5% dextrose-0.45% saline solution). The rate of EDTA infusion was adjusted to produce a linear decrease in arterial Ca^{2+} concentration from 60 to 90 minutes. A hypocalcemic clamp, in which Ca^{2+} concentrations were kept at 90-minute values, was maintained from 90 to 120 minutes.[7,13] During the induction of hypocalcemia, the EDTA infusion rate was progressively increased from 0.19 to 0.57 mmol/kg/h. During the hypocalcemic clamp in the period between 90 and 120 minutes, the infusion rate was progressively decreased from 0.57 to 0.15 mmol/kg/h. The total dose of EDTA infused was 0.303 mmol/kg. To prevent hypomagnesemia during the EDTA infusion (60 to 120 minutes), a solution of magnesium sulfate was infused at an increasing rate (0.200 to 0.375 mmol/kg/h) from 60 to 90 minutes, and then the rate was reduced to 0.1 mmol/kg/h during the hypocalcemic clamp (total dose of magnesium infused, 0.225 mmol/kg).

Experimental protocol for group II—Metabolic acidosis was induced in 10 dogs via IV infusion of HCl (2.5 mEq/kg in 200 mL of distilled water) during the 120-minute study. The rate of HCl infusion was 2.25 mEq/kg/h (from 0 to 60 minutes), 1.75 mEq/kg/h (from 60 to 90 minutes), and 1.25 mEq/kg/h (from 90 to 120 minutes; total dose, 3.75 mEq of HCl/kg). During the first 60 minutes, EDTA was infused IV at 0.08 mmol/kg/h to prevent an acidosis-induced increase in arterial Ca^{2+} concentration and maintain Ca^{2+} concentration within reference limits (normocalcemic clamp). Because the infusion of EDTA resulted in hypomagnesemia, magnesium was infused at a rate of 0.05 mmol/kg/h to maintain normalcy. From 60 to 90 minutes, hypocalcemia was induced by a progressively increasing dose of EDTA. Then, to maintain a hypocalcemic clamp (90 to 120 minutes), a progressively decreasing dose of EDTA was infused. Plasma magnesium concentration was maintained within reference limits (1.1 to 1.45 mEq of magnesium/L) by use of a magnesium infusion. The total doses of EDTA and magnesium were 0.432 and 0.3 mmol/kg, respectively.

Experimental protocol for group III—For a period of 60 minutes, metabolic acidosis was induced in 10 dogs via the same experimental protocol as that used in group II, but EDTA was not infused to control the arterial Ca^{2+} concentration. As a result, the Ca^{2+} concentration increased during the induction of metabolic acidosis. After 60 minutes, the study was ended and hypocalcemia was not induced. During the 60-minute infusion of HCl, the total dose was 2.25 mmol of HCl/kg. Because EDTA was not administered, plasma magnesium concentrations did not change.

Experimental protocol for group IV—Five dogs underwent an experimental protocol identical to that used in group II for the first 90 minutes except that before starting the experiment, the thyroid-parathyroid glands were surgically exposed and sutures were placed loosely around the thyroid vessels. At 90 minutes (end of induction of hypocalcemia), a parathyroidectomy was performed.[8] Briefly, the preplaced sutures were tightened and the thyroid-parathyroid glands were removed in < 5 minutes. In all dogs, 4 parathyroid glands were identified in the resected tissue. A hypocalcemic clamp was maintained from 90 to 120 minutes.

Assessments—Systolic and diastolic blood pressures were measured at the times of blood sample collection in all groups. Concentrations of ionized calcium, sodium, potassium, and chloride and values of pHa, Pa_{O_2}, and Pa_{CO_2} were measured in arterial blood samples with selective electrodes[l]; measurements were performed immediately after each sample was obtained. By use of an immunoradiometric assay,[m] the concentration of intact PTH in arterial blood was quantified. The use of this assay for measurement of PTH concentration in dogs has been validated previously.[7,8,13,14] Parathyroid hormone values for each dog were measured in 1 assay. The arterial bicarbonate concentration was calculated from the Pa_{CO_2} and pHa values by use of the Henderson-Hasselbach equation. Plasma phosphate and magnesium concentrations were measured via spectrophotometry.[n,o] At the end of the experiments, dogs were euthanized with an overdose of sodium thiopental.

Statistical analysis—For normally distributed data (all parameters except arterial Ca^{2+} and PTH concentrations), the unpaired Student *t* test was used for the comparison of 2 groups at the same time interval. A 1-way ANOVA was used to compare more than 2 groups and, if the ANOVA test revealed a significant difference (significance set at a value of $P < 0.05$), a post hoc Scheffe test was performed to determine intergroup differences. A repeated-measures ANOVA, followed by the post hoc Scheffe test, was used to compare more than 2 means from the same experimental group. For Ca^{2+} and PTH concentrations, nonparametric testing (ie, Mann-Whitney, Friedman ANOVA, and Wilcoxon tests) was used. A Pearson test was performed to determine the correlation between systolic blood pressure and Pa_{O_2} and between sys-

tolic blood pressure and ionized calcium values. In the metabolic acidotic dogs with and without the normocalcemic clamp (group II and group III, respectively), a direct comparison of PaO_2 values at each time interval by an unpaired *t* test did not identify differences between the 2 groups. However, at each time interval during the 60-minute comparison, the PaO_2 value was less in group III than in group II; therefore, the effect on PaO_2 was evaluated over the entire 60-minute period. This evaluation was performed by use of an ANCOVA in which the response variable was PaO_2, the covariates were pH and arterial Ca^{2+} concentration, and the factor analyzed was the difference between groups II and III. For all statistical tests, a value of $P < 0.05$ was considered significant. Results are presented as mean ± SE values.

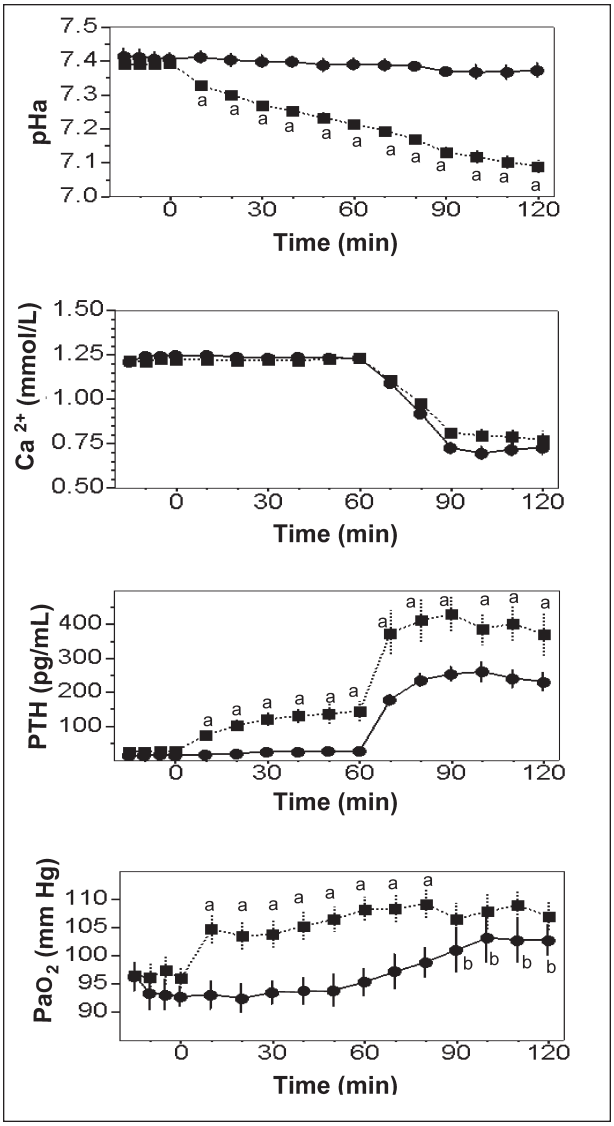

Figure 1—Changes in pHa, arterial Ca^{2+} concentration, PTH concentration, and PaO_2 in 10 anesthetized dogs in which metabolic acidosis was induced at time 0 and hypocalcemia was induced after 60 minutes of normocalcemic conditions (group II;squares) versus data for 8 dogs in which normal acid-base status was maintained and hypocalcemia was induced after 60 minutes of normocalcemic conditions (group I;circles). The letter a indicates that the value in group II is significantly ($P < 0.05$) different from that in group I at the same time point. The letter b indicates that the value in group I at this time point was significantly ($P < 0.05$) different from the 60-minute value.

Results

Arterial Ca^{2+} and PTH concentrations, pHa, and PaO_2 values in group I (ie, dogs in which normal acid-base status was maintained) and group II (ie, acidotic dogs in which hypocalcemia was induced after 60 minutes of normocalcemia) were compared (**Figure 1**). The infusion of HCl in group II dogs resulted in a progressive decrease in pHa values that reached 7.21 ± 0.02 at 60 minutes and 7.09 ± 0.02 at 120 minutes (both values were significantly [$P < 0.001$] different from baseline value). Because EDTA was infused to prevent increases in arterial Ca^{2+} concentration, Ca^{2+} values did not increase in group II during the first 60 minutes of the experimental period. Thus, at all time intervals from 0 to 60 minutes, arterial Ca^{2+} concentrations were not significantly different between groups I

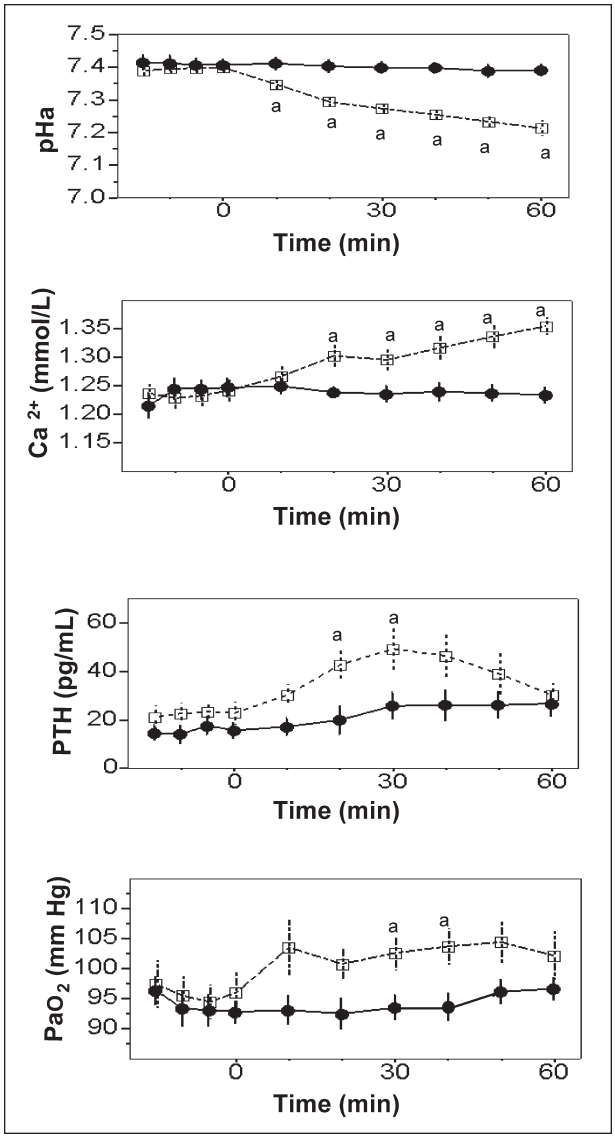

Figure 2—Changes in pHa, arterial Ca^{2+} concentration, PTH concentration, and PaO_2 in 10 anesthetized dogs in which metabolic acidosis was induced at time 0 and hypercalcemia was allowed to develop during a 60-minute period (group III;squares) versus data for 8 dogs in which normal acid-base status and normocalcemia were maintained during a similar period (group I; closed circles). *See* Figure 1 for key.

and II. From 60 to 90 minutes, hypocalcemia was induced with an EDTA infusion in both groups; arterial Ca^{2+} concentration decreased similarly in both groups (decrease of approx 0.4mM), followed by similar values during the hypocalcemic clamp (90 to 120 minutes). Parathyroid hormone concentrations did not change in group I from 0 to 60 minutes, but in group II, the values increased significantly ($P < 0.01$) from a baseline concentration of 27 ± 4 pg/mL to 146 ± 30 pg/mL at 60 minutes.

During the first 60 minutes in group II, the induction of metabolic acidosis resulted in an increase ($P < 0.01$) in PaO_2 values from a baseline value of 96 ± 2 mm Hg to 108 ± 2 mm Hg. The increase in PaO_2 was evident by 10 minutes (at a pHa value of 7.33 ± 0.001). Subsequent reductions in pHa from 7.33 to 7.21 at 60 minutes did not result in any further increase in PaO_2. At all sampling times between 10 and 60 minutes, PaO_2 values in group II were significantly greater than the baseline value of that group; also, group II values were significantly greater than group I values at the same time intervals (Figure 1). During the induction of hypocalcemia (from 60 to 90 minutes) in group I, PaO_2 values increased ($P < 0.02$) from 95 ± 2 mm Hg to 104 ± 3 mm Hg and then remained increased during application of the hypocalcemic clamp (90 to 120 minutes). However, in contrast to the rapid increase in PaO_2 associated with acidosis in group II, the hypocalcemia-induced increase in PaO_2 in group I was slow and progressive.

In the dogs with metabolic acidosis in which arterial Ca^{2+} concentration was not clamped (group III), pHa values decreased from 7.40 ± 0.02 to 7.21 ± 0.02 at 60 minutes. At all sampling times from 10 to 60 minutes, pHa values in group III were significantly less than baseline value of that group; also, group III values were significantly lower than group I values at the same time intervals (**Figure 2**). Because EDTA was not administered during the induction of acidosis, arterial Ca^{2+} concentration increased progressively from a baseline value of 1.24 ± 0.02mM to a maximum of 1.36 ± 0.02mM at 60 minutes. From 20 to 60 minutes, Ca^{2+} values in group III were greater ($P < 0.05$)

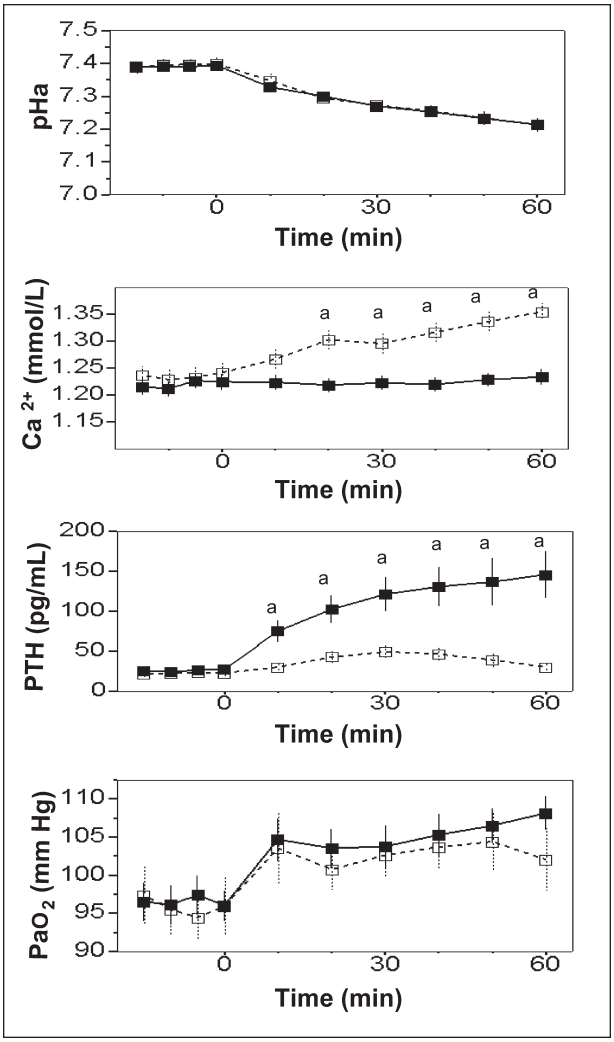

Figure 3—Changes in pHa, arterial Ca^{2+} concentration, PTH concentration, and PaO_2 in 10 anesthetized dogs in which metabolic acidosis was induced at time 0 and normocalcemia was maintained during a 60-minute period (group II; closed squares) versus data for 10 anesthetized dogs in which metabolic acidosis was induced at time 0 and hypercalcemia was allowed to develop during a similar period (group III; open squares). The letter a indicates that the value for group II was significantly ($P < 0.05$) different from that for group III at this time point.

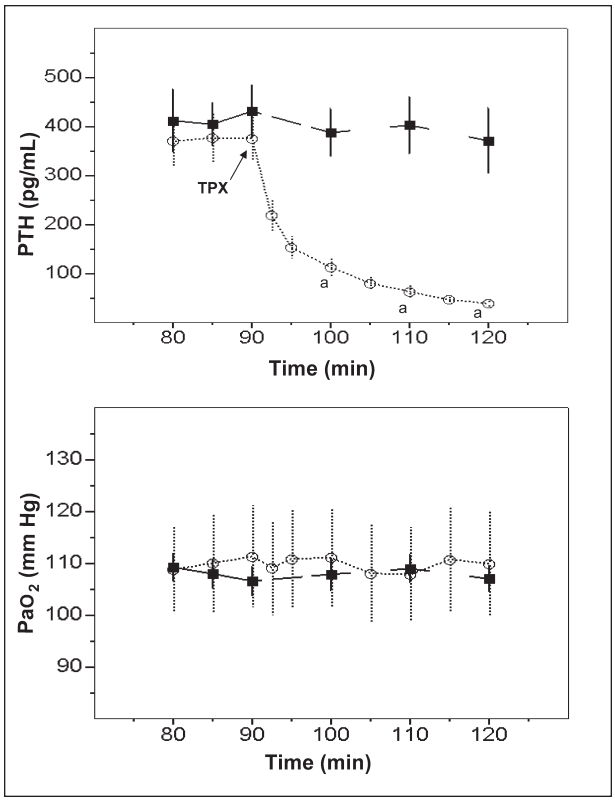

Figure 4—Changes in PTH concentration and PaO_2 in 5 dogs in which metabolic acidosis was induced at time 0, hypocalcemia was induced after 60 minutes of normocalcemic conditions, and thyroparathyroidectomy (TPX) was performed at 90 minutes (group IV; open circles) versus data for 10 anesthetized dogs in which metabolic acidosis was induced at time 0 and hypocalcemia was induced after 60 minutes of normocalcemic conditions (parathyroid glands remained intact; group II; closed squares). The letter a indicates that the value in group IV is significantly ($P < 0.05$) different from that in group II at the same time point.

than the baseline value; also, group III values were significantly ($P < 0.05$) greater than group I values at the same time intervals. In group III, PTH concentration increased from baseline values (23 ± 5 pg/mL) in response to acidosis and reached a maximum of 49 ± 9 pg/mL at 30 minutes. However, as the arterial Ca^{2+} concentration continued to increase after 30 minutes, PTH concentration started to decrease, and by 60 minutes, the value was not greater than baseline or the 60-minute value in group I. An increase in Pao_2 was also detected in group III after the first 10 minutes of acidosis, and at 30 and 40 minutes, the values were significantly ($P < 0.05$) greater than the corresponding values in group I.

The comparison between the acidotic dogs with and without the calcium clamp (group II and group III, respectively) revealed that the decreases in pHa during 60 minutes in those groups were not different (**Figure 3**). Arterial Ca^{2+} concentrations were greater and PTH values were less in group III than in group II. Compared with group II, Pao_2 values were less in group III at every time interval but were not significantly different at any time interval. Because of the consistency of the lesser mean Pao_2 values at all time intervals in group III, an ANCOVA (which integrates the effect on Pao_2 values during the entire 60 minutes) was performed. That analysis revealed that the Pao_2 value was less ($P < 0.01$) in group III than in group II when integrated for the entire 60 minutes of study. However, the integrated difference in Pao_2 between groups II and III was small, being only 2 mm Hg.

In dogs that underwent parathyroidectomy at 90 minutes of the experimental period (group IV), PTH values rapidly decreased during the subsequent 30 minutes (**Figure 4**). Parathyroidectomy did not affect pHa or arterial Ca^{2+} concentration because these were controlled by external infusions. In group IV dogs, Pao_2 values were unaffected by parathyroidectomy and remained high from 90 to 120 minutes. In that interval (90 to 120 minutes), there were no differences in Pao_2 between the parathyroidectomized dogs and group II dogs, which were similarly acidotic and hypocalcemic.

Blood pressure (systolic and diastolic) values at baseline were similar among the groups (**Table 1**). In

Table 1—Results (mean ± SE) of arterial blood gas and plasma biochemical analyses and blood pressure measurements at baseline (0 min) and after 60, 90, and 120 minutes of the experimental period in 3 groups of anesthetized dogs.

		Time (min)			
Variable	**Group**	**0**	**60**	**90**	**120**
Pao_2 (mm Hg)	I[a]	93.0 ± 1.9	95.4 ± 2.1	103.7 ± 3.1†	103.8 ± 2.3†
	II[b]	96.0 ± 2.1	108.2 ± 2.3*†	106.6 ± 2.9†	107.0 ± 2.5†
	III[c]	96.0 ± 3.7	102.1 ± 4.1		
$Paco_2$ (mm Hg)	I	34.9 ± 1.3	35.7 ± 1.2	36.9 ± 1.7	36.9 ± 1.7
	II	34.6 ± 0.7	34.6 ± 0.6	34.4 ± 0.6	33.9 ± 0.6
	III	33.4 ± 1.3	34.4 ± 1.2		
Bicarbonate (mEq/L)	I	21.6 ± 0.9	21.3 ± 1.0	21.0 ± 1.1	21.6 ± 1.3
	II	20.7 ± 0.4	13.7 ± 0.5*†	11.3 ± 0.5*†	10.2 ± 0.5*†
	III	20.1 ± 0.7	13.7 ± 0.9*†		
Sodium (mEq/L)	I	142.9 ± 1.8	143.3 ± 1.7	143.3 ± 1.8	142.7 ± 2.0
	II	142.0 ± 1.5	141.1 ± 1.2	140.4 ± 1.2	138.7 ± 0.9
	III	142.7 ± 0.8	142.5 ± 0.7		
Potassium (mEq/L)	I	4.1 ± 0.1	3.9 ± 0.1	3.6 ± 0.1†	3.5 ± 0.1†
	II	4.1 ± 0.1	4.0 ± 0.1	3.9 ± 0.1	4.1 ± 0.3
	III	4.3 ± 0.1	4.3 ± 0.1		
Chloride (mEq/L)	I	109.5 ± 1.2	109.8 ± 1.2	108.1 ± 0.9	108.7 ± 0.9
	II	111.7 ± 0.9	116.1 ± 0.8*†	116.1 ± 0.7*†	116.7 ± 0.9*†
	III	108.1 ± 0.9	112.6 ± 0.8*†		
Magnesium (mEq/L)	I	1.34 ± 0.07	1.33 ± 0.07	1.26 ± 0.02	1.35 ± 0.05
	II	1.21 ± 0.04	1.20 ± 0.04	1.21 ± 0.03	1.25 ± 0.05
	III	1.34 ± 0.04	1.32 ± 0.07		
Phosphate (mg/dL)	I	3.4 ± 0.4	3.6 ± 0.4	3.2 ± 0.3	3.0 ± 0.2
	II	3.4 ± 0.2	3.6 ± 0.3	3.0 ± 0.3	3.0 ± 0.4
	III	3.6 ± 0.6	3.9 ± 0.9		
BP (S/D) (mm Hg)	I	156 ± 9/81±5	155 ± 9/84±5	118 ± 10/65±7†	128 ± 11/71±7
	II	161 ± 8/84±3	163 ± 7/88±5	151 ± 6/79±3*	150 ± 7/83±3
	III	152 ± 11/78±7	148 ± 13/81±6		

*Value significantly ($P < 0.05$) different from that for group I (control) at same time point. †Value significantly ($P < 0.05$) different from baseline value (0 minutes).
BP (S/D) = Blood pressure (systolic/diastolic).
[a]Anesthetized dogs (n = 8) in which normal acid-base status was maintained and hypocalcemia was induced after 60 minutes of normocalcemic conditions. [b]Anesthetized dogs (n = 10) in which metabolic acidosis was induced at time 0 and hypocalcemia was induced after 60 minutes of normocalcemic conditions. [c]Anesthetized dogs (n = 10) in which metabolic acidosis was induced at time 0 and hypercalcemia was allowed to develop over a period of 60 minutes.

group I, hypocalcemia resulted in a significant ($P < 0.05$) decrease in blood pressure from 156 ± 9 mm Hg to 118 ± 10 mm Hg (systolic) and from 81 ± 5 mm Hg to 65 ± 7 mm Hg (diastolic) at 90 minutes. The decrease in systolic blood pressure during the induction of hypocalcemia (from 60 to 90 minutes) strongly correlated with the increase in Pa_{O_2} values ($r = –0.66$; $P < 0.001$) and had a weaker correlation with the decrease in arterial Ca^{2+} concentration ($r = 0.43$; $P < 0.01$). The decreased blood pressure did not change at 120 minutes during the hypocalcemic clamp. No significant changes in systolic blood pressure were observed after 60 minutes of acidosis in either group II or group III. The blood pressure values in group II during hypocalcemia at 90 and 120 minutes were not different from baseline.

The Pa_{CO_2} value and sodium, phosphate, and magnesium concentrations in arterial blood did not differ at any time point within the same group or among the different groups. As expected, bicarbonate concentration decreased and chloride concentration increased in groups II and III. A small but significant decrease in potassium concentration was detected in dogs during the induction of hypocalcemia in group I. At all time points, values of Pa_{O_2} and Pa_{CO_2} and concentrations of bicarbonate, sodium, potassium, chloride, magnesium, and phosphate for dogs in group IV (that underwent the same experimental protocol as group II except that parathyroidectomy was performed at 90 minutes to determine the role of PTH) did not differ from those in group II.

Discussion

In the present study, the induction of hypocalcemia increased the Pa_{O_2} value in dogs. Moreover, the increase in Pa_{O_2} correlated with a decrease in systolic blood pressure during the induction of hypocalcemia. In addition to verifying that the induction of metabolic acidosis increases Pa_{O_2} values, our data have indicated that the increase in arterial Ca^{2+} concentration induced by metabolic acidosis attenuated the acidosis-induced increase in Pa_{O_2}.

The effect of metabolic acidosis on Pa_{O_2} in dogs has been determined previously. Haas and Bergofsky[11] reported a decrease in the alveolar-to-arterial O_2 gradient in anesthetized dogs in which acidosis was rapidly induced. Those investigators attributed the increase in Pa_{O_2} to a more homogeneous distribution of ventilation to perfusion in the lung. This hypothesis was challenged by results of subsequent studies,[9,10] which indicated that the acidosis-induced increase in Pa_{O_2} was mainly a consequence of a rightward shift of the oxyhemoglobin dissociation curve. Our results also have indicated that metabolic acidosis increases the Pa_{O_2} value by a magnitude similar to that previously reported.[9–11]

The induction of hypocalcemia was associated with a slow, progressive increase in Pa_{O_2} that correlated with a decrease in systolic blood pressure, which in turn correlated with a decrease in arterial Ca^{2+} concentration. In anesthetized dogs, hypocalcemia has been reported[15,16] to reduce cardiac output and decrease peripheral vascular resistance, both of which would act to decrease blood pressure. In dogs, resting pulmonary vascular tone is low and is not lowered further by potent vasodilators.[17] Drop et al[18] reported that pulmonary vessels are not sensitive to changes in arterial Ca^{2+} concentration. However, Farrukh and Michael[19] determined that decreased Ca^{2+} concentration does attenuate hypoxemia-induced pulmonary vasoconstriction. Our data, obtained in the absence of hypoxemia, indicated that the induction of hypocalcemia correlated with an increase in Pa_{O_2} and a decrease in systolic blood pressure in dogs. The decrease in blood pressure induced by hypocalcemia is associated with decreased cardiac output.[15,20–22] Also, because the dogs were receiving assisted ventilation with fixed F_{IO_2} and tidal volume and had the same value of Pa_{CO_2}, it seems unlikely that there was a change in alveolar gas concentration. Intrapulmonary shunt is also known to vary directly with cardiac output.[23] Thus, the best explanation for the increase in Pa_{O_2} associated with hypocalcemia appears to be that the decrease in cardiac output reduced the pulmonary shunt by reducing blood flow through underventilated areas of the lung.

It is also important to note that blood pressure did not change during the induction of hypocalcemia in dogs with metabolic acidosis in the present study. Several factors associated with acidosis might be responsible for the maintenance of normal blood pressure. These include an increase in the contractile force of the left ventricle mediated by sympathoadrenal factors and a catecholamine-mediated arterial and venous vasoconstriction that enhanced blood delivery to the central circulation and counteracted the decrease in peripheral vascular resistance induced by acidosis.[24]

In the acidotic dogs with and without hypercalcemia, a direct comparison at each time interval did not reveal a significant difference in Pa_{O_2} values; nevertheless, at each time interval, the Pa_{O_2} value was less in the dogs with acidosis and hypercalcemia. Consequently, an ANCOVA was performed to evaluate the effect over the 60-minute period and revealed a modest, but significant, decrease in Pa_{O_2} in the dogs with acidosis and hypercalcemia, compared with the dogs with acidosis and normocalcemia. One possible explanation for the lower Pa_{O_2} value in acidotic and hypercalcemic dogs is that hypercalcemia increases cardiac contractility and output,[20,21,25] which in turn has been associated with increased pulmonary shunting.[23,26–28]

The lesser increase in Pa_{O_2} during the acidosis-induced increase in arterial Ca^{2+} concentration together with the increase in Pa_{O_2} detected during the period of hypocalcemia suggests that serum calcium concentration affects the Pa_{O_2} value. Although the increase in Pa_{O_2} in acidotic dogs and hypocalcemic dogs was similar (change in Pa_{O_2} values of approx 10 mm Hg), the increase in Pa_{O_2} was slowly progressive during the induction of hypocalcemia, whereas a small decrease in pHa resulted in a maximal increase in Pa_{O_2}. With regard to the acidosis-induced increase in arterial Ca^{2+} concentration, the small Pa_{O_2} differences between normo- and hypercalcemic acidotic dogs would seem to have limited impact on blood oxygenation and consequently be of little clinical relevance. Increases in Pa_{O_2} induced by metabolic acidosis or hypocalcemia

may have clinical relevance in the acute care setting, but studies need to be performed to evaluate their importance in critically ill dogs in which acidosis, hypocalcemia, and reduced cardiac output frequently develop. In clinically normal or noncritically ill dogs undergoing anesthesia, the development of metabolic acidosis or reductions in arterial Ca^{2+} concentration may affect Pa_{O_2} values and thus require adjustment of ventilatory settings.

Other factors that change in parallel with alterations in Ca^{2+} concentration might also have affected the Pa_{O_2} value. One such factor is PTH, which increases in concentration as Ca^{2+} concentration and pHa values decrease. Parathyroid hormone has cardiovascular effects. Results of several studies[29,30] have determined that the infusion of PTH has a hypotensive effect, but chronic excess of PTH has been associated with systemic[31] and pulmonary[32] hypertension. Because both acidosis and hypocalcemia increase PTH concentration and Pa_{O_2}, it is possible that changes in PTH concentration were responsible for the Pa_{O_2} changes detected in the dogs of the present study. However, this possibility seems unlikely because Pa_{O_2} values continued to increase during hypocalcemia even after maximal PTH concentrations were obtained and the acidosis-induced increase in Pa_{O_2} values was attenuated during the period of hypercalcemia induced by acidosis even though PTH values did not change. Finally and most importantly, the increased Pa_{O_2} values in acidotic dogs were unaffected by parathyroidectomy, even though PTH values decreased rapidly as acidosis and hypocalcemia were maintained. Thus, we do not believe that PTH contributed to the changes in Pa_{O_2}.

Another factor to consider is the administration of EDTA, which was used to induce the hypocalcemia in the dogs of the present study. The progressive hypocalcemia was induced by increasing the amount of EDTA administered. However, during application of the hypocalcemic clamp, the EDTA dose was reduced 4-fold, and this did not result in a decrease in Pa_{O_2}. Thus, any effect of EDTA on Pa_{O_2} would seem to be indirect and mediated by hypocalcemia.

In our study, maintenance of dogs on mechanical ventilation minimized changes in Pa_{CO_2}. In the absence of fixed ventilation, the induction of metabolic acidosis does result in compensatory hyperventilation with a decrease in Pa_{CO_2}, which should have 2 opposite effects on values of Pa_{O_2}; there would likely be an increase in Pa_{O_2} mediated by the increase in alveolar P_{O_2} and a lesser decrease in pHa, which would act to minimize an acidosis-induced increase in Pa_{O_2}.

Our data have indicated that the induction of hypocalcemia increases the Pa_{O_2} value of anesthetized dogs by a mechanism that is independent of PTH and is likely related to the cardiovascular effects of hypocalcemia that probably resulted in decreased pulmonary shunting. Furthermore, results of the present study in dogs have indicated that the acidosis-induced increase in Pa_{O_2} values is modestly reduced by the increase in arterial Ca^{2+} concentration induced by metabolic acidosis. The effect of metabolic acidosis and hypocalcemia on Pa_{O_2} should be taken into account in anesthetized dogs in which these abnormalities develop. Whether the improved oxygenation detected in healthy dogs during the induction of metabolic acidosis and hypocalcemia would occur in critically ill dogs remains to be determined.

a. Ketolar 50 mg, Parke-Davis SL, Barcelona, Spain.
b. Fentanest, Productos Roche SA, Madrid, Spain.
c. Thalamonal, Productos Roche SA, Madrid, Spain.
d. Pentotal sodico 1 g, Abbot Laboratories, Madrid, Spain.
e. Servoventilador, Siemens-Elema 900, Lycksele, Sweden.
f. Dormicum, Productos Roche SA, Madrid, Spain.
g. Pavulon, Organon Teknika Española, Barcelona, Spain.
h. Hewlett Packard 7754B system, Houston, Tex.
i. HCl 37%, Merck KgaA, Darmstadt, Germany.
j. Na_2EDTA, Sigma-Aldrich Chemie Gmbh, Steinheim, Germany.
k. Na_2SO_4, Panreac, Barcelona, Spain.
l. Ciba-Corning Model 850, Bayer Diagnostics, Barcelona, Spain.
m. Nichols Institute Diagnostics, San Juan Capistrano, Calif.
n. Phosphorus inorganic, Sigma Diagnostics, St Louis, Mo.
o. Magnesium, Sigma Diagnostics, St Louis, Mo.

References

1. King LG, Wohl JS, Manning AM, et al. Evaluation of the survival prediction index as a model of risk stratification for clinical research in dogs admitted to intensive care units at four locations. *Am J Vet Res* 2001;62:948–954.

2. Vaden SL, Levine J, Breitschwerdt EB. A retrospective case-control of acute renal failure in 99 dogs. *J Vet Intern Med* 1997; 11:58–64.

3. Macintire DK. Emergency therapy of diabetic crisis: insulin overdose, diabetic ketoacidosis, an hyperosmolar coma. *Vet Clin North Am Small Anim Pract* 1995;25:639–650.

4. Gronert GA, Haskins SC, Steffey EP. Plasma electrolyte and metabolite concentrations associated with pentobarbital or pentobarbital-propofol anesthesia during three weeks' mechanical ventilation and intensive care in dogs. *Lab Anim Sci* 1998; 48:513–519.

5. Otto KA, Weber BP, Jacobi M, et al. Retrospective evaluation of cardiopulmonary and acid-base variables during long-term balanced anesthesia for experimental surgery in dogs. *Lab Anim Sci* 1997;47:624–631.

6. Cote CJ. Depth of halothane anesthesia potentiates citrate-induced ionized hypocalcemia and adverse cardiovascular events in dogs. *Anesthesiology* 1987;67:676–680.

7. Lopez I, Aguilera-Tejero E, Estepa JC, et al. Role of acidosis-induced increases in calcium on PTH secretion in acute metabolic and respiratory acidosis. *Am J Physiol Endocrinol Metab* 2004; 286:E780–E785.

8. Lopez I, Aguilera-Tejero E, Felsenfeld AJ, et al. Direct effect of acute metabolic and respiratory acidosis on parathyroid hormone secretion in the dog. *J Bone Miner Res* 2002;17:1691–1700.

9. Frans A, Clerbaux T, Willems E, et al. Effect of metabolic acidosis on pulmonary gas exchange of artificially ventilated dogs. *J Appl Physiol* 1993;74:2301–2308.

10. Frans A, Turek Z, Yokota H, et al. Effect of variation in blood hydrogen ion concentration on pulmonary gas exchange of artificially ventilated dogs. *Pflugers Arch* 1979;380:35–39.

11. Haas F, Bergofsky EH. Effect of pulmonary vasoconstriction on balance between alveolar ventilation and perfusion. *J Appl Physiol* 1968;24:491–497.

12. National Research Council. *Guide for the care and use of laboratory animals*. Washington, DC: National Academy Press, 1996.

13. Estepa JC, Aguilera-Tejero E, Almaden Y, et al. Effect of calcium reduction and a hypocalcemic clamp on parathyroid hormone secretion: a study in dogs. *Kidney Int* 1999;55:1724–1733.

14. Torrance AG, Nachreiner R. Human-parathormone assay for use in dogs: validation, sample handling studies, and parathyroid function testing. *Am J Vet Res* 1989;50:1123–1127.

15. Drop LJ, Geffin GA, O'Keefe DD, et al. Relation between ionized calcium concentration and ventricular pump performance in the dog under hemodynamically controlled conditions. *Am J Cardiol* 1981;47:1041–1051.

16. Scheidegger D, Drop LJ, Schellenberg JC. Role of the systemic vasculature in the hemodynamic response to changes in plasma ionized calcium. *Arch Surg* 1980;115:206–211.

17. Kadowitz PJ, Nandiwada P, Gruetter CA, et al. Pulmonary vasodilator responses to nitroprusside and nitroglycerin in the dog. *J Clin Invest* 1981;67:893–902.

18. Drop LJ, Toal KW, Geffin GA, et al. Pulmonary vascular responses to hypercalcemia and hypocalcemia in the dog. *Anesthesiology* 1989;70:825–836.

19. Farrukh IS, Michael JR. Cellular mechanisms that control pulmonary vascular tone during hypoxia and normoxia. Possible role of Ca^{2+}ATPases. *Am Rev Respir Dis* 1992;145:1389–1397.

20. McLean FC, Hastings AB. A biological method for the estimation of calcium ion concentration. *J Biol Chem* 1934;107:337–350.

21. Raines AEG, Seymour A-ML, Roberts AFC, et al. Impairment of cardiac function and energetics in experimental renal failure. *J Clin Invest* 1993;92:2934–2940.

22. Stultz PM, Scheidegger D, Drop LJ, et al. Ventricular pump performance during hypocalcemia: clinical and experimental studies. *J Thorac Cardiovasc Surg* 1979;78:185–194.

23. Wagner PD, Schaffartzik W, Prediletto R, et al. Relationship among cardiac output, shunt, and inspired O_2 concentration. *J Appl Physiol* 1991;71:2191–2197.

24. Kraut JA, Kurtz I. Use of base in the treatment of severe acidemic states. *Am J Kidney Dis* 2001;38:703–727.

25. Bristow MR, Schwartz HD, Binetti G, et al. Ionized calcium and the heart. Elucidation of in-vivo concentration-response relationships in the open chested dog. *Circ Res* 1977; 41:565–574.

26. Eldridge MW, Dempsey JA, Haverkamp HC, et al. Exercise-induced intrapulmonary arteriovenous shunting in healthy humans. *J Appl Physiol* 2004;97:797–805.

27. Hopkins SR, Barker RC, Brutsaert TD, et al. Pulmonary gas exchange during exercise in women: effects of exercise type and work increment. *J Appl Physiol* 2000;89:721–730.

28. Stickland MK, Welsh RC, Haykowsky MJ, et al. Intra-pulmonary shunt and pulmonary gas exchange during exercise in humans. *J Physiol* 2004;561:321–329.

29. Baksi SN. Hypotensive action of parathyroid hormone in hypoparathyroid and hyperparathyroid rats. *Hypertension* 1988;11:509–513.

30. Togari A, Sintani S, Arai M, et al. Acute effect of parathyroid hormone (1–34) fragment on blood pressure in rats fed a low calcium diet. *Gen Pharmacol* 1990;21:547–549.

31. Jespersen B, Randlov A, Abrahamsen J, et al. Effects of PTH (1–34) on blood pressure, renal function, and hormones in essential hypertension. *Am J Hypertens* 1997;10:1356–1367.

32. Akmal M, Barndt RR, Ansari AN, et al. Excess PTH in CRF induces pulmonary calcification, pulmonary hypertension and right ventricular hypertrophy. *Kidney Int* 1995;47:158–163.