Role of acidosis-induced increases in calcium on PTH secretion in acute metabolic and respiratory acidosis in the dog

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López, Ignacio, Escolástico Aguilera-Tejero, José Carlos Estepa, Mariano Rodríguez, and Arnold J. Felsenfeld. Role of acidosis-induced increases in calcium on PTH secretion in acute metabolic and respiratory acidosis in the dog. Am J Physiol Endocrinol Metab 286: E780 –E785, 2004. First published January 13, 2004; 10.1152/ajpendo.00473.2003.—Recently, we showed that both acute metabolic acidosis and respiratory acidosis stimulate parathyroid hormone (PTH) secretion in the dog. To evaluate the specific effect of acidosis, ionized calcium (iCa) was clamped at a normal value. Because iCa values normally increase during acute acidosis, we now have studied the PTH response to acute metabolic and respiratory acidosis in dogs in which the iCa concentration was allowed to increase (nonclamped) compared with dogs with a normal iCa concentration (clamped). Five groups of dogs were studied: control, metabolic (clamped and nonclamped), and respiratory (clamped and nonclamped) acidosis. Metabolic (HCl infusion) and respiratory (hyperventilation) acidosis was progressively induced during 60 min. In the two clamped groups, iCa was maintained at a normal value with an EDTA infusion. Both metabolic and respiratory acidosis increased (P < 0.05) iCa values in nonclamped groups. In metabolic acidosis, the increase in iCa was progressive and greater (P < 0.05) than in respiratory acidosis, in which iCa increased by 0.04 mM and then remained constant despite further pH reductions. The increase in PTH values was greater (P < 0.05) in clamped than in nonclamped groups (metabolic and respiratory acidosis). In the nonclamped metabolic acidosis group, PTH values first increased and then decreased from peak values when iCa increased by >0.1 mM. In the nonclamped respiratory acidosis group, PTH values exceeded (P < 0.05) baseline values only after iCa values stopped increasing at a pH of 7.30. For the same increase in iCa in the nonclamped groups, PTH values increased more in metabolic acidosis. In conclusion, 1) both metabolic acidosis and respiratory acidosis stimulate PTH secretion; 2) the physiological increase in the iCa concentration during the induction of metabolic and respiratory acidosis reduces the magnitude of the PTH increase; 3) in metabolic acidosis, the increase in the iCa concentration can be of sufficient magnitude to reverse the increase in PTH values; and 4) for the same degree of acidosis-induced hypercalcemia, the increase in PTH values is greater in metabolic than in respiratory acidosis.

besides its well-known effect on calcium homeostasis, parathyroid hormone (PTH) has also been associated with changes in the acid-base status. In 1970, Wills (26) suggested that PTH-induced bone resorption mobilized phosphate to act as a buffer to neutralize acidosis. In subsequent studies, PTH administration has been shown to increase net acid excretion (14, 24) and induce metabolic alkalosis in dogs and humans by both renal and extrarenal mechanisms (15–17). Because PTH appears to have a role in acid-base homeostasis, it would seem reasonable that acid-base disorders may affect PTH secretion. We recently showed that both acute metabolic acidosis and respiratory acidosis increase PTH secretion (18). To determine the specific effect of acute metabolic and respiratory acidosis on PTH secretion, the ionized calcium concentration was clamped at a normal value. But in the absence of a calcium clamp, both acute metabolic acidosis and respiratory acidosis increase the ionized calcium concentration (4, 22). Previous studies have shown that, for the same decrease in blood pH, the increase in ionized calcium is greater in metabolic than in respiratory acidosis (4, 22). Because both acute metabolic acidosis and respiratory acidosis normally increase the ionized calcium concentration (4, 22), the increase in calcium may reduce the magnitude of PTH stimulation by metabolic and respiratory acidosis. Moreover, because the ionized calcium increase is greater in metabolic acidosis, any reduction in PTH values might be greater in metabolic acidosis. Thus, during acidosis, the parathyroids may receive two signals acting in opposite directions: stimulation from acidosis and inhibition from hypercalcemia.

Our goal was to evaluate in a dog model of acute metabolic and respiratory acidosis, the extent to which acidosis-induced increases in the ionized calcium concentration modify the increase in PTH values.

METHODS

Animals

Healthy mongrel dogs, ages 2–5 yr with a mean weight of 26 ± 4 kg, were used for study. Five groups of dogs were studied: control, metabolic acidosis with and without calcium clamp, and respiratory acidosis with and without calcium clamp. Details on the care, feeding, medications, sedation, placement of intravenous catheters, intubation, anesthesia, ventilation, and blood pressure monitoring are provided in a recent publication (18).

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 Science and published by the National Institutes of Health (NIH; NIH publ-

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cation no. 86-23, revised 1985). The experimental protocols were reviewed and approved by The Ethics Committee for Animal Research of the Universidad de Cordoba (Spain).

To establish baseline values for each group, blood samples were obtained four times during a 20-min observation period. During the experimental period of 60 min, the ionized calcium concentration was clamped at a normal value by an EDTA infusion in one of the two groups with metabolic acidosis and with respiratory acidosis. During the 60-min study, blood was drawn at 10-min intervals. Results from dogs in the two clamped groups have been reported previously (18).

**Experimental Groups**

Controls. Dogs (n = 10) were followed for 60 min without modification of their acid-base status. During the experiment, the dogs received an infusion of 5% dextrose in 0.45% NaCl solution and were ventilated at 12 breaths/min (tidal volume = 15 ml·kg⁻¹·h⁻¹).

Metabolic acidosis. Metabolic acidosis was induced by the intravenous infusion of HCl (2.5 meq/kg in 200 ml of water) in two groups of dogs, which were ventilated at 12 breaths/min. The rate of infusion was 2.25 mmol·kg⁻¹·h⁻¹. The total amount of HCl infused was 2.25 mmol/kg. To prevent an acidosis-induced increase in the ionized calcium concentration, EDTA was infused in one group (n = 8) at 0.08 mmol·kg⁻¹·h⁻¹ during the 60 min. In the other group (n = 7), the ionized calcium concentration was allowed to increase during the induction of metabolic acidosis. An intravenous infusion of magnesium (0.05 mmol·kg⁻¹·h⁻¹) was needed to prevent hypomagnesemia in the group receiving EDTA.

Respiratory acidosis. Respiratory acidosis was induced by hyperventilation in two groups (n = 7 in each group). Tidal volume was reduced from 15 to 10 ml/kg and the respiratory rate from 12 to 8 breaths/min. To prevent the development of hypoxemia from hyperventilation, the P_FiO₂, was increased to 30%. These parameters were maintained throughout the study. To prevent an acidosis-induced increase in the ionized calcium concentration, EDTA was infused intravenously in one of the two groups. An intravenous infusion of magnesium (0.0375 mmol·kg⁻¹·h⁻¹) was needed to prevent hypomagnesemia in the group receiving EDTA.

The total volume of intravenous fluids given was adjusted by the infusion of a D5 half-normal saline solution so that the amount of fluid received was identical among the five groups.

Ionized calcium, pH, sodium, P_CO₂, and P_O₂ were measured with selective electrodes (Ciba-Corning Diagnostics, Madrid, Spain), and measurements were performed immediately after the blood sample was obtained. The bicarbonate concentration was calculated from the P_CO₂ and pH values by use of the Henderson-Hasselbach equation. Plasma phosphate and magnesium were measured by spectrophotometry. Intact PTH was measured with an immunoradiometric assay (Nichols, San Juan Capistrano, CA). The use of this assay for the measurement of PTH in the dog has been validated previously (1, 10, 11, 23, 25).

Moreover, in a recent study in the normal dog (12), we have shown that a good correlation exists between the intact [measures only PTH-(1–84)] and large amino-truncated fragments] and the whole [measures only PTH-(1–84)] PTH assays. To prevent interassay variation in PTH values, all of the samples obtained in one dog during the 60-min study period were measured in the same assay for PTH.

**Statistics**

For the comparison of two samples, the paired or unpaired Student t-test was used. One-way ANOVA was used to compare more than two groups, and when a difference was present, a post hoc test, the Fish error least significant difference test, was used to determine intergroup differences. Repeated-measures ANOVA was used to compare more than two means from the same experimental group. An ordinal regression analysis was performed to determine whether the slopes of two regression lines were different. A P value of <0.05 was considered significant. Results are shown as means ± SE.

**RESULTS**

During the 60-min study, blood pH values did not change in the control group (Fig. 1). The linear decrease in blood pH values was similar between the metabolic and respiratory acidosis groups, both in the two nonclamped (Fig. 1A) and the two clamped (Fig. 1B) groups.

In the two nonclamped groups, a similar increase in calcium concentration (Δcalcium) was observed by 10 min (Fig. 2A). The subsequent ionized calcium values increased progressively during the induction of metabolic acidosis, but in the respiratory acidosis group ionized calcium values did not increase after the first 10–20 min despite further reductions in the blood pH. By design, the ionized calcium concentration did not change in the two clamped groups (Fig. 2B).

In the nonclamped group with metabolic acidosis, ionized calcium values increased progressively as the blood pH was lowered. When all the data points from the dogs in this group were included, the correlation between the change in ionized calcium and the decrease in blood pH was r = −0.88, P < 0.001. In the nonclamped group with respiratory acidosis, this same correlation was r = −0.54, P < 0.001. Because the ionized calcium value in this group did not increase further after the first 10–20 min despite additional reductions in the blood pH, the same comparison was made for only the first 20 min, and the correlation was r = −0.78, P < 0.001. The slope of the regression line was steeper in metabolic acidosis than in respiratory acidosis: −0.494 ± 0.032 vs. −0.327 ± 0.043, P < 0.001.
In the nonclamped groups, a difference in the pattern of increase in PTH was observed between the metabolic and respiratory acidosis groups (Fig. 3A). In the metabolic acidosis group, PTH values increased from baseline by 20 min \((P < 0.05)\) and remained so until 40 min, after which PTH values declined as ionized calcium values increased further. In the respiratory acidosis group, PTH values did not increase from baseline until 50 min and remained increased at 60 min. The progressive increase in PTH was observed even though ionized calcium values did not change between 10 and 60 min (Fig. 2A). PTH values increased in the clamped groups by 10 min and remained so during the remainder of the study (Fig. 3B). There were no significant differences in PTH values between metabolic and respiratory acidosis in the two clamped groups.

Because of small differences in blood pH at the lower pH values between the clamped and nonclamped groups of metabolic acidosis and those of respiratory acidosis, the \(\Delta\text{calcium}\) and PTH values were stratified for blood pH to facilitate the comparison. Figure 4 shows the comparison of changes in \(\Delta\text{calcium}\) and PTH values between nonclamped and clamped groups with metabolic acidosis. In the nonclamped group, there was a progressive increase in the ionized calcium concentration in response to the lowering of blood pH (Fig. 4A). Associated with the increase in the calcium concentration, the stimulatory effect of metabolic acidosis on PTH secretion was reduced. Thus, in the nonclamped group, PTH values increased \((P < 0.05)\) from baseline at pH values from 7.30 to 7.26. Subsequent decreases in pH resulted in a decline in PTH in the nonclamped group to PTH values not different from baseline.

As shown in Table 1, plasma sodium, bicarbonate, magnesium, and phosphate values were measured at baseline (0 min) were greater \((P < 0.05)\) in the clamped group than in the nonclamped group in the pH ranges 7.34–7.30 and 7.24–7.22 (Fig. 4B).

A comparison of the changes in blood pH, calcium, and PTH in the two respiratory acidosis groups is shown in Fig. 5. In the clamped group, PTH values increased \((P < 0.05)\) from baseline by a pH of 7.34 and remained elevated at lower pH values (Fig. 5B). In the nonclamped group, PTH values increased more slowly for the same changes in pH and were significantly greater than baseline only between pH values of 7.26 and 7.22 (Fig. 5B). The failure to find an increase in PTH in the nonclamped group until a pH value of 7.26 was associated with an increasing ionized calcium concentration until a pH of 7.30 (Fig. 5A). As the pH value was lowered from 7.30 to 7.22, PTH values increased in the nonclamped group as ionized calcium levels did not increase further. During the same pH reductions in the clamped group, PTH values remained unchanged but greater than those in the nonclamped group.

The change in PTH values for a similar increase in ionized calcium is shown for the two nonclamped acidosis groups in Fig. 6. Because ionized calcium values increased progressively during 60 min in the metabolic acidosis group but not in the respiratory acidosis group, the range of ionized calcium values was wider in the metabolic acidosis group. In the range of hypercalcemic values common to both groups, higher PTH levels were observed in the metabolic acidosis group. At the ionized calcium concentrations at which differences in PTH values were observed, blood pH values were not different.

As shown in Table 1, plasma sodium, bicarbonate, magnesium, and phosphate values were measured at baseline (0 min)
and at the end of the study (60 min). At baseline, there were no differences among the groups for any biochemical measure-
ment. At 60 min, differences were observed only for plasma bicarbonate. In the two respiratory acidosis groups, bicarbonate
values were greater than in the control group, whereas in the two metabolic acidosis groups values were less than in the
control group. Also in the two metabolic acidosis groups, the bicarbonate value at 60 min was less than that at baseline.

DISCUSSION

In a recent study (18), we evaluated the effect of metabolic and respiratory acidosis on PTH secretion when the calcium
concentration was clamped at a normal value. The present study shows that the physiological increase in the ionized

![Fig. 4](image1)

**Fig. 4.** Comparison of the Δcalcium (A) and ΔPTH (B) changes vs. blood pH in the metabolic acidosis groups (clamped vs. nonclamped). *P < 0.05 vs. baseline; **P < 0.05 vs. nonclamped group.

![Fig. 5](image2)

**Fig. 5.** Comparison of the Δcalcium (A) and ΔPTH (B) changes vs. blood pH in the respiratory acidosis groups (clamped vs. nonclamped). *P < 0.05 vs. baseline; **P < 0.05 vs. nonclamped group.

![Fig. 6](image3)

**Fig. 6.** PTH-calcium relationship during acidosis-induced hypercalcemia in the nonclamped metabolic (solid bars) and respiratory (dotted bars) acidosis groups. *P < 0.05 vs. respiratory acidosis.

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**DISCUSSION**

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concentration was clamped at a normal value. The present study shows that the physiological increase in the ionized

![Table 1](image4)

**Table 1.** Plasma sodium, bicarbonate, magnesium, and phosphate values at baseline (0 min) and 60 min

<table>
<thead>
<tr>
<th>Plasma</th>
<th>Time, min</th>
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<th>60</th>
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<tr>
<td>Sodium, meq/l</td>
<td>Control</td>
<td>142.2 ± 1.4</td>
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<td></td>
<td>Met acid clamp</td>
<td>142.4 ± 1.4</td>
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<td>Resp Acid clamp</td>
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<td></td>
<td>Met Acid no clamp</td>
<td>142.2 ± 1.1</td>
<td>142.2 ± 0.8</td>
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<td></td>
<td>Resp Acid no clamp</td>
<td>144.8 ± 1.2</td>
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<tr>
<td>Bicarbonate, meq/l</td>
<td>Control</td>
<td>21.6 ± 0.7</td>
<td>21.0 ± 0.7*</td>
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<td></td>
<td>Met Acid clamp</td>
<td>21.9 ± 0.7</td>
<td>14.8 ± 0.5**</td>
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<td>23.3 ± 0.6</td>
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<td>12.7 ± 1.0**</td>
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<tr>
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<td>24.6 ± 1.2*</td>
</tr>
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<td>Magnesium, meq/l</td>
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<td>1.41 ± 0.04</td>
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<tr>
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<td>Resp Acid clamp</td>
<td>1.34 ± 0.03</td>
<td>1.38 ± 0.03</td>
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<tr>
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<td>1.32 ± 0.06</td>
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<tr>
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<td>Resp Acid no clamp</td>
<td>1.33 ± 0.06</td>
<td>1.28 ± 0.04</td>
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<tr>
<td>Phosphate, mg/dl</td>
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<td>3.4 ± 0.3</td>
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<tr>
<td></td>
<td>Met Acid clamp</td>
<td>3.5 ± 0.3</td>
<td>3.5 ± 0.3</td>
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<td></td>
<td>Resp Acid clamp</td>
<td>3.5 ± 0.2</td>
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<td></td>
<td>Met Acid no clamp</td>
<td>3.6 ± 0.6</td>
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</tr>
<tr>
<td></td>
<td>Resp Acid no clamp</td>
<td>3.7 ± 0.5</td>
<td>4.8 ± 0.5</td>
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Values are means ± SE. Met, metabolic; Resp, respiratory; Acid, acidosis.

One-way ANOVA was used to determine differences among groups, and differences were observed only for plasma bicarbonate at 60 min (*P < 0.001). Intragroup differences for plasma bicarbonate at 60 min were determined by post hoc test. *P < 0.05 vs. 0 min. **P < 0.05 vs. a. ***P < 0.05 vs. a and b.
calcium concentration that occurs during the induction of both metabolic and respiratory acidosis reduces, but does not eliminate, the acidosis-induced increase in PTH secretion.

The difference in the PTH response between clamped and nonclamped groups in metabolic and respiratory acidosis was likely due to the suppressive effect of calcium countering the acidosis-induced stimulation of PTH. It is also important to recognize that an increase in the calcium concentration normally results in PTH suppression. Thus, when PTH values increase marginally or remain unchanged while calcium values increase, such a result should not be interpreted as a lack of an effect of acidosis. Rather, the lack of change in PTH values should be compared with the suppressive effect on PTH values of a similar 0.1 mM increase in ionized calcium concentration.

In previous studies in dogs, such an increase in calcium concentration decreased PTH values by 20–30 pg/ml to near-maximal suppression (1, 23).

As was shown previously (18), the increase in PTH values in the clamped groups during the induction of acute metabolic and respiratory acidosis was similar. In both conditions, a decrease in blood pH of −0.10 units resulted in near-maximal PTH stimulation. The acidosis-induced stimulation of PTH is approximately one-fourth of the maximal PTH stimulation seen with hypocalcemia (1, 10, 18, 23).

In metabolic acidosis, the most likely interpretation for our results is that the increase in the ionized calcium concentration acted to reduce the increase in PTH values. When the increase in ionized calcium values surpassed −0.1 mM, it even began to reverse the stimulatory effect of acidosis on PTH values. Moreover, because the maximal acidosis-induced stimulation of PTH secretion was reached at a pH of 7.30, any further increase in ionized calcium below a pH of 7.30 should enhance the suppressive effect of calcium. In respiratory acidosis, the failure of ionized calcium values to continue to increase, and perhaps the lesser magnitude of calcium increase compared with metabolic acidosis, would seem to have allowed the stimulatory effect of respiratory acidosis on PTH secretion to prevail. Finally, the dynamic of an increasing ionized calcium concentration followed by a static level may also have contributed to the observed PTH response in the nonclamped group (5).

The stimulation of PTH in the nonclamped groups was greater in metabolic than in respiratory acidosis. It could be argued that the greater PTH response during acidosis-induced hypercalcemia is not in agreement with the similar PTH increase seen in the clamped metabolic and respiratory acidosis groups. But then, during the induction of hypocalcemia, we have previously reported that the PTH response was greater in metabolic than in respiratory acidosis (18). It is possible that the greater PTH response seen in metabolic acidosis during the induction of hypo- and hypercalcemia may be explained by the fact that the rapid diffusion of CO2 between the extra- and intracellular compartments in respiratory acidosis results in a more rapid equilibration of pH (13, 19), which in turn may have the potential to modify the calcium-sensing mechanism.

In previous studies, PTH administration has been shown to generate a metabolic alkalosis (15–17). Moreover, hypercalcemia independent of PTH has also been reported to generate a metabolic alkalosis (6, 21). Thus acidosis-induced PTH stimulation and the resultant hypercalcemia may act together to correct acidosis by reducing bicarbonate excretion and increasing net acid excretion by the kidney (14). Moreover, the acidosis-induced increase in PTH probably enhances the release of bone buffer (3). Furthermore, the increased phosphate release from bone and renal phosphate excretion induced by both acidosis (2) and the increase in PTH values would act to promote net acid excretion (14). Thus it would seem likely that the acidosis-induced PTH stimulation and hypercalcemia contribute to the correction of acidosis.

Acute metabolic and respiratory acidosis are often seen in critically ill patients admitted to intensive care units (7, 28). Moreover, hypocalcemia is often observed despite the presence of acidosis in these patients, and the hypocalcemia is associated with an increased mortality (7, 28) and may also adversely affect the blood pressure (8). Whether acute acidosis in this setting acts to stimulate PTH secretion to maintain a normal calcium concentration and enhance net acid excretion is difficult to know because of the many associated confounding variables, such as hypomagnesemia, altered vitamin D metabolism, hyperphosphatemia, and renal failure, that are often present (27). Another potential role for metabolic acidosis, which may potentiate the effect of PTH and thus prevent hypocalcemia in acute metabolic acidosis, is the increased uptake of PTH by bone (20) and the recently described up-regulation of the PTH/PTH-related protein receptor (9). The results of our study at least suggest the interesting possibility that acidosis in critically ill patients might have the potential to stimulate PTH secretion, which in turn might help to maintain a normal calcium concentration and also to correct the acidosis.

In conclusion, our study shows that 1) both metabolic acidosis and respiratory acidosis stimulate PTH secretion; 2) the physiological increase in ionized calcium concentration during the induction of metabolic and respiratory acidosis reduces the magnitude of the acidosis-induced increase in PTH values; 3) in metabolic acidosis, the increase in the ionized calcium concentration can be of sufficient magnitude to reverse the increase in PTH values; and 4) for the same degree of acidosis-induced hypercalcemia, the increase in PTH values is greater in metabolic than in respiratory acidosis.

GRANTS

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