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Metabolic, but not respiratory, acidosis increases bone PGE_2 levels and calcium release

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Bushinsky, David A., Walter R. Parker, Kristen M. Alexander, and Nancy S. Krieger. Metabolic, but not respiratory, acidosis increases bone PGE_2 levels and calcium release. *Am J Physiol Renal Physiol* 281: F1058–F1066, 2001. First published July 12, 2001; 10.1152/ajprenal.00355.2001.—A decrease in blood pH may be due to either a reduction in bicarbonate concentration ($[HCO_3^-]$; metabolic acidosis) or to an increase in P_{CO_2} (respiratory acidosis). In mammals, metabolic, but not respiratory, acidosis increases urine calcium excretion without altering intestinal calcium absorption, indicating that the additional urinary calcium is derived from bone. In cultured bone, chronic metabolic, but not respiratory, acidosis increases net calcium efflux (J_{Ca}), decreases osteoblastic collagen synthesis, and increases osteoclastic bone resorption. Metabolic acidosis increases bone PGE_2 production, which is correlated with J_{Ca}, and inhibition of PGE_2 production inhibits this acid-induced J_{Ca}. Given the marked differences in the osseous response to metabolic and respiratory acidosis, we hypothesized that incubation of neonatal mouse calvariae in medium simulating respiratory acidosis would not increase medium PGE_2 levels, as observed during metabolic acidosis. To test this hypothesis, we determined medium PGE_2 levels and J_{Ca} from calvariae incubated at pH ~7.1 to model either metabolic (Met; $[HCO_3^-]$ ~11 mM) or respiratory (Resp; P_{CO_2} ~83 Torr) acidosis, or at pH ~7.5 as a control (Ntl). We found that after 24–48 and 48–51 h in culture, periods when cell-mediated J_{Ca} predominates, medium PGE_2 levels and J_{Ca} were increased with Met, but not Resp, compared with Ntl, and there was a direct correlation between medium PGE_2 levels and J_{Ca}. Thus metabolic, but not respiratory, acidosis induces the release of bone PGE_2, which mediates J_{Ca} from bone.

calvariae; osteoblasts; protons; pH

IN HUMANS AND EXPERIMENTAL ANIMALS, chronic metabolic acidosis, a decrease in systemic pH induced by a decrease in serum bicarbonate concentration ($[HCO_3^-]$), increases urine calcium excretion (15, 49, 51) without increasing intestinal calcium absorption (50, 51), resulting in a negative calcium balance (15, 50, 51). Because >98% of total body calcium is contained within the bone mineral (83), this negative calcium balance strongly implies a loss of bone calcium. Indeed, in humans dietary intake of acid precursors causes an apparent decrease in bone mineral content, which is reversed by the provision of alkali (50, 67).

In contrast, chronic respiratory acidosis, a decrease in systemic pH induced by an increase in P_{CO_2}, appears to have little (30) or no (48, 65, 66) effect on urine calcium excretion. Clearly, any effect on urine calcium excretion is far less than that induced by metabolic acidosis (30). Serum calcium may increase slightly during respiratory acidosis (48). The lack of an appreciable change in urine calcium excretion during respiratory, compared with metabolic, acidosis suggests a marked difference in the osseous response to these two types of acidosis (10).

We have extensively compared the response of cultured bone to a similar degree of metabolic and respiratory acidosis (16). During short-term (3-h) incubations, both types of acidosis cause a net calcium efflux from bone; however, isohydric metabolic acidosis causes a far greater net efflux than does respiratory acidosis (22, 27, 28, 31). Respiratory acidosis not only causes less unidirectional calcium efflux but has been shown to cause deposition of medium calcium onto bone (28). Over this short time period, these changes are due to alterations in the physicochemical driving forces for bone mineralization and dissolution (20, 23). The low total CO_2 concentration during metabolic acidosis favors dissolution, whereas high total CO_2 concentration during respiratory acidosis favors formation of the carbonated apatite in bone (27). The additional protons present in the medium during metabolic acidosis are buffered by the bone mineral whereas there is little buffering of protons during respiratory acidosis (5, 6, 18, 21). The proton buffering during metabolic acidosis is apparently due, at least in part, to the depletion of bone carbonate that occurs during metabolic, but not respiratory, acidosis (22, 23).

During longer term incubations, there is a significant cell-mediated component of calcium efflux from bone during models of metabolic, but not respiratory, acidosis (7, 11, 47). Although we have shown that metabolic acidosis increases the resorptive activity of

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osteoclasts, as measured by increased β-glucuronidase release, and inhibits osteoid synthesis by osteoblasts, as measured by decreased collagen synthesis and alkaline phosphatase activity, isohydric respiratory acidosis has little effect on these parameters of bone cell activity (11, 47). Primary bone cells, principally osteoblasts and osteoblast precursors, differentiate and mineralize in culture (2, 3, 33, 69). Metabolic acidosis inhibits mineralization to a greater extent than does respiratory acidosis (70). Utilizing a high-resolution, scanning ion microprobe to examine the ion composition of bone, we have shown that metabolic acidosis causes a greater efflux of bone sodium than calcium and bone potassium than calcium whereas respiratory acidosis has little effect on the bone mineral (14, 24, 28, 31).

We have examined the effects of metabolic acidosis on the RNA levels of several genes known to be expressed in osteoblasts. After acute stimulation with serum, metabolic acidosis selectively inhibits expression of *Egr-1* and type 1 collagen RNA compared with stimulation at neutral medium pH (38). In contrast, expression of *c-fos*, *c-jun*, *junB*, and *junD* RNA were not affected by a similar decrement in medium pH. In chronic bone cell cultures maintained up to 6 wk, metabolic acidosis inhibited expression of matrix Gla protein and osteopontin RNA relative to expression in neutral medium (36). Expression of osteonectin, transforming growth factor-β, and glyceraldehyde-3-phosphate dehydrogenase were not affected by acidosis. However, in subsequent experiments we found that RNA levels for osteopontin and matrix Gla protein were inhibited to a similar extent by both chronic metabolic and respiratory acidosis, suggesting that their genes are not responsible for the observed differences in bone cell function (37). RNA levels for osteonectin were not affected by either type of acidosis.

In nonosseus cells, metabolic acidosis increases the levels of prostaglandins (1, 34, 35, 41). In both toad bladder (34, 35) and rat kidney (41), PGE_2 levels have been shown to increase in response to metabolic acidosis. In the brains of newborn pigs, acidosis increases the level of prostaglandins that are associated with vasodilatation (1). An increase in prostaglandin levels by bone cells is important as prostaglandins are potent local stimulators of bone resorption and appear to mediate resorption induced by a variety of cytokines and growth factors (35, 62). Goldhaber and Rabadjija (40) first demonstrated that the prostaglandin inhibitor indomethacin inhibits acid-induced, cell-mediated calcium efflux from bone, and Rabadjija et al. (61) subsequently demonstrated that protons stimulate release of PGE_2 from neonatal mouse calvariae. We recently demonstrated that incubation of bone cells in medium simulating metabolic acidosis led to an increase in the level of medium PGE_2 (46) and that incubation of calvariae in similarly acidic medium led to a parallel increase in PGE_2 levels and net calcium efflux.

Given the marked differences in the osseous response to metabolic and respiratory acidosis, we hypothesized that incubation of neonatal mouse calvariae in medium simulating respiratory acidosis would not lead to the increase in medium PGE_2 levels that is observed during metabolic acidosis (46). The results presented here support this hypothesis; we found that metabolic, but not respiratory, acidosis increased bone culture medium PGE_2 levels and net bone calcium release. There was a strong, direct, correlation between bone culture medium PGE_2 levels and net calcium release. Thus metabolic, but not respiratory, acidosis induces the release of bone PGE_2 that appears to mediate net calcium release.

METHODS

Organ Culture of Bone

Exactly 2.8 ml of DMEM containing 15% heat-inactivated horse serum were preincubated at a fixed, chosen P_{CO_2} (either 40 or 85 Torr) at 37°C for 3 h in 35-mm dishes (5–7, 11, 13, 17–25, 27–29, 31, 36–38, 46, 47, 70). Calvariae were dissected from 4- to 6-day-old neonatal mice, and, just before the bones were added, 1 ml of medium was removed to determine preincubation medium pH, P_{CO_2}, and calcium. Medium pH and P_{CO_2} were determined with a blood-gas analyzer (Radiometer model ABL 5) and calcium by electrode (Nova Biomedical). We compared this electrode to the fluorometric titrator (Calcette, Precision Systems) that we had used previously (6, 7, 11, 13, 17–25, 27–29, 31, 36–38, 46, 47, 70) and found excellent agreement [$r = 0.985$, $n = 18$, $P = <0.001$, electrode Ca = 1.05 × (fluorometric Ca) + 0.23]. Calvariae were incubated for an initial 24 h and were then moved to fresh, similarly preincubated medium for an additional 24 h; they were again moved to fresh, similarly preincubated medium for an additional 3 h. At the beginning and end of each incubation period, the medium was removed and analyzed for pH, P_{CO_2}, and calcium. After the 24- to 48- and 48- to 51-h incubation periods, the medium was also immediately analyzed for PGE_2. [HCO_3^-] was calculated from medium pH and P_{CO_2} as described previously (6, 21). Net calcium flux was calculated as $V_m([Ca]_f - [Ca]_i)$, where V_m is the medium volume (1.8 ml), and $[Ca]_f$ and $[Ca]_i$ are the final and initial medium calcium concentrations, respectively.

Treatment Groups

Calvariae were divided into three groups. Calvariae were incubated in medium either at neutral pH (7.5; Ntl), acidic pH (7.1) produced by a decrease in the [HCO_3^-] to model metabolic acidosis (Met), or an increase in the P_{CO_2} to model respiratory acidosis (Resp). To closely replicate physiological conditions only the HCO_3^-/CO_2 buffer system was used (12). To model Met, concentrated HCl was added to the Ntl medium (5 μl /2.8 ml medium), resulting in a reduction of [HCO_3^-] and, thus, pH. To model Resp, the P_{CO_2} of Ntl medium was increased by increasing the CO_2 concentration in the incubator. Five microliters of deionized distilled water (per 2.8 ml of medium) were added to the Ntl and Resp incubations.

Prostaglandin Levels

The level of medium PGE_2 in the calvariae was determined immediately after the end of the incubation with the use of an enzyme immunoassay kit (Cayman Chemical, Ann Arbor, MI). Quantitation of the assay was done by using a Dynatech

MR700 microplate reader and the Immunosoft computer program.

Statistical Analysis

All values are expressed as means ± SE. Tests of significance were calculated by analysis of variance with the Bonferroni correction for multiple comparisons and regression analysis using conventional computer programs (BMDP; University of California, Los Angeles, CA). $P < 0.05$ was considered significant.

RESULTS

Medium pH, P_{CO_2}, and $[HCO_3^-]$

To determine whether PGE_2 levels are increased in respiratory as well as metabolic acidosis and to determine whether any increase in PGE_2 levels are responsible for acid-induced calcium release from bone, we incubated calvariae under neutral pH or acidic conditions simulating metabolic and respiratory acidosis. During each of the three time periods, 0–24, 24–48, and 48–51 h, the initial medium pH was significantly lower in both Met and Resp than in Ntl (Table 1). There was no difference in initial medium pH between Met and Resp during any of the three time periods. During each of the three time periods, the initial medium $[HCO_3^-]$ was lower in the Met compared with the Resp and Ntl groups, and the initial medium P_{CO_2} was higher in the Resp compared with the Met and Ntl groups.

During each of the three time periods, 0–24, 24–48, and 48–51 h, the final medium pH was significantly lower in both Met and Resp than in Ntl, and the final medium pH in the Met group was lower than that in the Resp group (Table 1). During each of the three time periods, the final medium $[HCO_3^-]$ was lower in the Met compared with Resp and Ntl, and the final medium P_{CO_2} was higher in the Resp compared with Met and Ntl.

Net Calcium Efflux and Medium PG Levels

0–24 H. At the conclusion of the initial 24-h incubation, compared with Ntl, incubation in Met medium led to a marked increase in net calcium efflux from calvariae (Table 1). Compared with Ntl there was no increase in net calcium efflux in Resp, and net calcium efflux from Met was greater than from Resp.

24–48 H. Although acid-induced calcium efflux during the first 24 h is due primarily to physicochemical calcium release (6, 20, 23, 29, 38), we have previously shown that calcium efflux in more prolonged incubations is due, primarily, to cell-mediated mechanisms (7, 11, 13, 14, 19, 25, 36, 37, 47, 70). At the conclusion of the subsequent 24-h incubation, incubation in Met led to a marked increase in net calcium efflux ($P < 0.001$) and an increase in medium PGE_2 concentration ($P < 0.001$) compared with culture in Ntl (Fig. 1). Compared with culture in Ntl, incubation in Resp did not lead to an increase in net calcium efflux [P = not significant (NS)] nor in medium PGE_2 concentration (P = NS). There was a significant inverse correlation between initial medium pH and net calcium flux in Ntl and Met combined ($r = -0.676$, $n = 23$, $P < 0.001$) but not in Ntl and Resp combined ($r = -0.245$, $n = 23$, P = NS). There was a significant inverse correlation between initial medium pH and medium PGE_2 concentration in Ntl and Met combined ($r = -0.735$, $n = 23$, $P < 0.001$) but not in Ntl and Resp combined ($r = -0.130$, $n = 23$, P = NS). Net calcium efflux and medium PGE_2 concentrations were greater in Met than in Resp (Met vs. Resp, each $P < 0.001$). During this time period there was a significant direct correlation between medium PGE_2 levels and net calcium flux in

Table 1. *Initial vs. final pH, P_{CO_2}, $[HCO_3^-]$, calcium and J_{Ca}*

Group	n	Initial pH	Initial P_{CO_2}, Torr	Initial $[HCO_3^-]$, meq/l	Initial Calcium, mg/dl	Final pH	Final P_{CO_2}, Torr	Final $[HCO_3^-]$, meq/l	J_{Ca}, nmol·bone^{-1}·24 h^{-1}
					0–24 h Incubation				
Ntl	12	7.462 ± 0.004	35.1 ± 0.2	24.9 ± 0.2	6.86 ± 0.02	7.305 ± 0.009	34.4 ± 0.5	16.6 ± 0.3	261 ± 37
Met	12	7.109 ± 0.007*	35.2 ± 0.1	10.9 ± 0.2*	6.87 ± 0.01	7.004 ± 0.012*	32.8 ± 0.7	7.8 ± 0.2*	620 ± 56*
Resp	12	7.120 ± 0.007*	82.8 ± 1.0*†	25.8 ± 0.2*†	6.90 ± 0.02	7.055 ± 0.009*†	80.5 ± 1.1*†	21.4 ± 0.2*†	350 ± 37†
					24–48 h Incubation				
Ntl	12	7.467 ± 0.006	34.7 ± 0.4	24.5 ± 0.1	6.85 ± 0.02	7.241 ± 0.018	35.8 ± 0.2	14.9 ± 0.5	
Met	12	7.120 ± 0.006*	34.1 ± 0.2	10.5 ± 0.1*	6.79 ± 0.02	6.889 ± 0.016*	34.8 ± 0.2	6.3 ± 0.3*	
Resp	12	7.115 ± 0.004*	82.6 ± 0.6*†	25.3 ± 0.1*†	6.80 ± 0.02	7.019 ± 0.011*†	84.0 ± 0.6*†	20.5 ± 0.5*†	
					48–51 h Incubation				
Ntl	12	7.457 ± 0.004	35.1 ± 0.2	24.4 ± 0.1	6.80 ± 0.03	7.408 ± 0.006	35.9 ± 0.6	21.9 ± 0.2	
Met	12	7.112 ± 0.004*	34.7 ± 0.2	10.8 ± 0.1*	6.78 ± 0.02	7.048 ± 0.005*	35.6 ± 0.2	9.3 ± 0.1*	
Resp	12	7.112 ± 0.003*	83.5 ± 0.5*†	25.4 ± 0.1*†	6.78 ± 0.02	7.073 ± 0.002*†	87.1 ± 0.4*†	24.4 ± 0.1*†	

Values are means ± SE of medium parameters. n, No. of calvariae; $[HCO_3^-]$, bicarbonate concentration; J_{Ca}, net calcium efflux. Neonatal mouse calvariae were incubated in neutral medium (Ntl) or in medium acidified by a primary reduction of $[HCO_3^-]$ to model metabolic acidosis (Met) or by a primary increase in P_{CO_2} to model respiratory acidosis (Resp) for an initial 24 h (0- to 24-h incubation). Calvariae were then moved to fresh, similarly preincubated medium for an additional 24 h (24- to 48-h incubation) and again moved to fresh, similarly preincubated medium for a additional 3 h (48- to 51-h incubation). *$P < 0.05$ vs. Ntl, same time period; †$P < 0.05$ vs. Met, same time period.

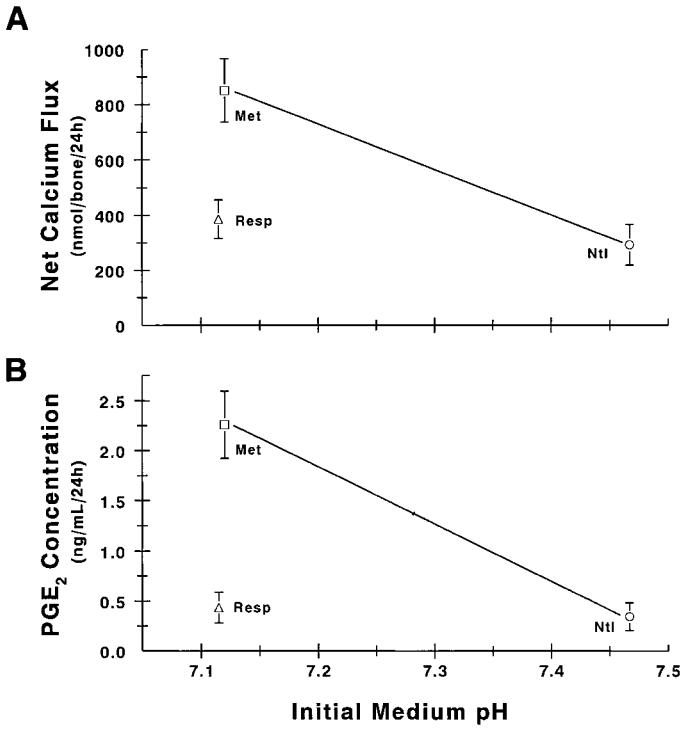

Fig. 1. Effect of acidosis on net calcium flux (*A*) and medium PGE_2 concentration (*B*) as a function of initial medium pH: 24- to 48-h time period. Neonatal mouse calvariae were incubated in neutral medium (Ntl) or in medium acidified by a primary reduction of the bicarbonate concentration to model metabolic acidosis (Met) or by a primary increase in P_{CO_2} to model respiratory acidosis (Resp) for an initial 24 h. Calvariae were then moved to fresh similar preincubated medium for an additional 24 h; data presented is for this latter 24-h incubation. Values are means ± SE. Compared with culture in Ntl, incubation in Met, but not in Resp, led to a significant increase in net calcium efflux and to a significant increase in medium PGE_2 concentration. Initial medium pH and net calcium flux were inversely correlated in Ntl and Met combined but not in Ntl and Resp combined. Initial medium pH and medium PGE_2 concentration were inversely correlated in Ntl and Met combined but not in Ntl and Resp combined. Net calcium efflux and medium PGE_2 concentrations were greater in Met than in Resp.

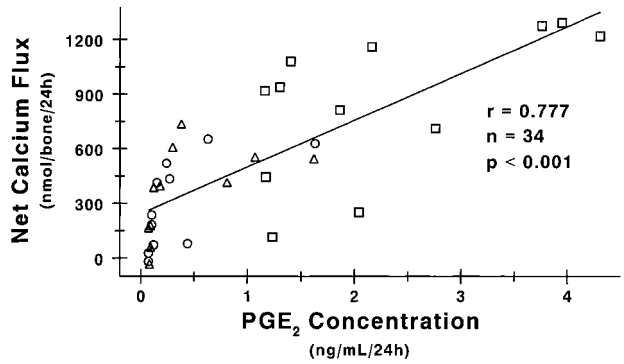

Fig. 2. Correlation between net calcium efflux and medium PGE_2 concentration: 24- to 48-h time period. Neonatal mouse calvariae were incubated in neutral medium (Ntl; ○) or in medium acidified by a primary reduction of the bicarbonate concentration to model metabolic acidosis (Met; □) or by a primary increase in P_{CO_2} to model respiratory acidosis (Resp; △) for an initial 24 h. Calvariae were then moved to fresh similar preincubated medium for an additional 24 h; data presented are for this latter 24-h incubation. During this time period there was a significant direct correlation between medium PGE_2 levels and net calcium flux in all groups (plotted) and in Ntl and Met combined but not Ntl and Resp combined.

all groups (Fig. 2) and in Ntl and Met combined (r = 0.784, n = 23, $P < 0.001$) but not in Ntl and Resp combined (r = 0.404, n = 23, P = NS).

48–51 H. At the conclusion of the subsequent 3-h incubation, compared with Ntl, incubation in Met led to a marked increase in net calcium efflux ($P < 0.001$) and an increase in medium PGE_2 concentration ($P <$ 0.001) (Fig. 3). Compared with culture in Ntl, incubation in Resp did not lead to an increase in net calcium efflux (P = NS) nor in medium PGE_2 concentration (P = NS). There was a significant inverse correlation between initial medium pH and net calcium flux in Ntl and Met combined ($r = -0.690$, n = 23, $P < 0.001$) but not in Ntl and Resp combined ($r = -0.331$, n = 23, P = NS). There was a significant inverse correlation between initial medium pH and medium PGE_2 concentration in Ntl and Met combined ($r = -0.638$, n = 22, P = 0.001) but not in Ntl and Resp combined (r = 0.010, n = 21, P = NS). Net calcium efflux and medium PGE_2 concentrations were greater in Met than in Resp

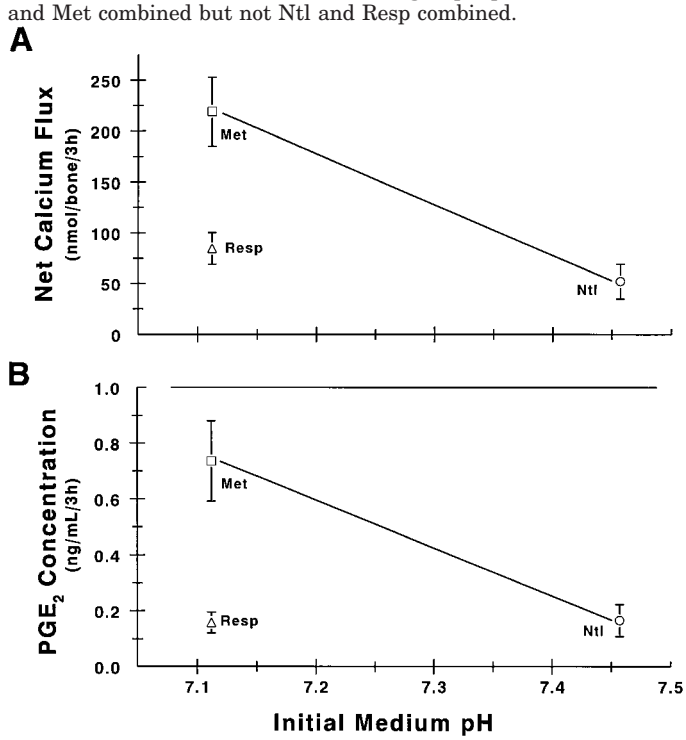

Fig. 3. Effect of acidosis on net calcium flux (*A*) and medium PGE_2 concentration (*B*) as a function of initial medium pH: 48- to 51-h time period. Neonatal mouse calvariae were incubated in neutral medium (Ntl) or in medium acidified by a primary reduction of the bicarbonate concentration to model metabolic acidosis (Met) or by a primary increase in P_{CO_2} to model respiratory acidosis (Resp) for an initial 24 h. Calvariae were then moved to fresh similar preincubated medium for an additional 24 h and again moved to fresh similar preincubated medium for an additional 3 h; data presented are for this final 3-h incubation. Values are means ± SE. Compared with culture in Ntl, incubation in Met, but not in Resp, led to a significant increase in net calcium efflux and to a significant increase in medium PGE_2 concentration. Initial medium pH and net calcium flux were inversely correlated in Ntl and Met combined but not in Ntl and Resp combined. Initial medium pH and medium PGE_2 concentration were inversely correlated in Ntl and Met combined but not in Ntl and Resp combined. Net calcium efflux and medium PGE_2 concentrations were greater in Met than in Resp.

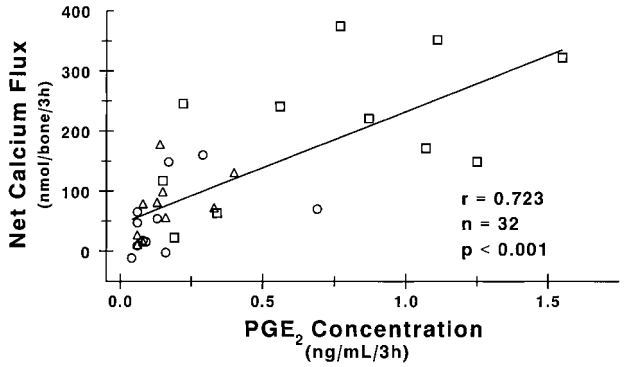

Fig. 4. Correlation between net calcium efflux and medium PGE_2 concentration: 48- to 51-h time period. Neonatal mouse calvariae were incubated in neutral medium Ntl (○) or in medium acidified by a primary reduction of the bicarbonate concentration to model metabolic acidosis (Met; □) or by a primary increase in P_{CO_2} to model respiratory acidosis (Resp; △) for an initial 24 h. Calvariae were then moved to fresh similar preincubated medium for an additional 24 h and again moved to fresh similar preincubated medium for a additional 3 h; data presented are for this final 3-h incubation. During this time period, there was a significant direct correlation between medium PGE_2 levels and net calcium flux in all groups (plotted) and in Ntl and Met combined but not Ntl and Resp combined.

(Met vs. Resp, each $P < 0.001$). During this time period there was a significant direct correlation between medium PGE_2 levels and net calcium flux in all groups (Fig. 4) and in Ntl and Met combined ($r = 0.717$, $n = 22$, $P < 0.001$) but not Ntl and Resp combined ($r = 0.386$, $n = 21$, $P =$ NS).

DISCUSSION

Metabolic acidosis leads to a marked increase in urine calcium excretion (15, 49, 51, 52). The source of this additional urinary calcium appears to be the mineral phases of bone (15, 50), as there is no increase in intestinal calcium absorption (39, 50). In contrast, there appears to be little (30), if any (48, 65, 66), increase in urine calcium excretion during respiratory acidosis. In vitro studies support these clinical observations (8–10, 16, 26). During models of metabolic acidosis, there is marked calcium efflux from cultured bone, depletion of bone carbonate, stimulation of osteoclastic resorption, inhibition of osteoblastic formation, and a decrease in the formation of mineralized bone nodules (5–7, 13, 14, 17, 20–25, 27–29, 31, 36, 38, 46, 47). However, isohydric respiratory acidosis causes far less calcium efflux, does not appear to affect bone carbonate or osteoblastic or osteoclastic function, and has far less effect on the formation and mineralization of bone nodules (6, 7, 11, 22, 27, 28, 31, 70).

When neonatal mouse bone is cultured in medium modeling metabolic acidosis, there is an increase in the level of PGE_2 in the medium (46, 61). PGE_2 has been shown to induce bone resorption (45, 77), and the observed acidosis-induced increase is of sufficient magnitude to induce resorption (46). The purpose of the present study was to test the hypothesis that incubation of neonatal mouse calvariae in medium simulating respiratory acidosis would not lead to the increase in medium PGE_2 levels that is observed during metabolic acidosis (46, 61). The results of this study strongly support our hypothesis. Medium PGE_2 levels are increased in metabolic, but not in isohydric respiratory, acidosis, and the increase is strongly correlated with net calcium efflux from bone.

Prostaglandins are potent multifunctional regulators of bone formation and resorption that mediate the response of bone to a variety of stimuli (62, 63). Prostaglandins, especially PGE_2, stimulate bone resorption in organ culture (45, 77) and regulate resorption of mouse calvariae in response to a variety of cytokines and growth factors, including epidermal growth factor (76), platelet-derived growth factor (75), tumor necrosis factor-α (TNF-α) (78), and transforming growth factor-β (TGF-β) (72, 79). In this study, as in our previous study (46), we chose to measure only PGE_2, as it is the most potent known metabolite of arachidonic acid that stimulates bone resorption in mouse calvariae (62, 77, 82).

The mechanism for the differential effect on medium PGE_2 of an isohydric decrement in pH produced by a decrease in medium HCO_3^- compared with an increase in P_{CO_2} was not addressed in this study. One potential mechanism for the differential stimulation of PGE_2 is the effect of these two types of acidosis on intracellular pH. Respiratory acidosis might be expected to lower cytosolic pH more rapidly than metabolic acidosis, as CO_2 is far more permeable across cell membranes than is HCO_3^-. Although we have not studied intracellular pH in mouse calvarial cells, we have previously determined the effects of models of metabolic and respiratory acidosis on the intracellular pH of UMR106 rat osteosarcoma cells (58). Within 1 min after exposure to acidosis, there is a more marked fall in intracellular pH with respiratory compared with metabolic, acidosis; however, after 24 and 48 h, the intracellular pH increased to normal with metabolic acidosis but continued to be suppressed with respiratory acidosis. That intracellular pH is reduced in chronic respiratory, but not in chronic metabolic, acidosis suggests that intracellular pH alone cannot be the mechanism by which PGE_2 is stimulated during metabolic acidosis.

A soluble adenylate cyclase (sAC) activity has been detected in cytosolic extracts from mammalian testis that is biochemically and chromatographically distinct from the better characterized transmembrane adenylyl cylcases (56). Its activity is dependent on Mn^{2+} and is insensitive to G protein regulation but is activated by sodium bicarbonate (54, 55, 57). The message for sAC is most abundant in male germ cells, suggesting that it plays a role in spermatozoa maturation, a process that is induced by HCO_3^-. The purification, molecular cloning, and functional expression of this unique signaling molecule in rat, mouse, and humans has recently been described by Buck et al. (4). This same group has also demonstrated that HCO_3^- regulates the activity of sAC both in vivo and in vitro in a pH-independent manner (32) and suggests that this enzyme is a HCO_3^- sensor important in mediating cAMP-dependent processes involved in sperm activation. It is possible that bone cells

also contain such a HCO_3^- sensor that helps mediate the response to metabolic acidosis.

We have previously shown that isolated bone cells, which are principally osteoblasts, are the source of the additional PGE_2 during metabolic acidosis (46). The differential effect on medium PGE_2 levels during the isohydric reduction of medium pH between metabolic and respiratory acidosis could be due to differences in osteoblastic prostaglandin production or degradation. Changes in prostaglandin levels are generally thought to be due to differences in production; however, a previous study has shown that degradation is altered as a function of pH (71). PGE_2 dehydrates in aqueous solution to PGA_2, and there is increased stability of PGE_2 in a model of metabolic acidosis compared with metabolic alkalosis (71). However, the difference in stability between pH 6 and 8 is only ~1.2%, far less than the difference between metabolic acidosis and neutral medium observed in this study, suggesting that differences in degradation were not the cause of the metabolic acidosis-induced increase in PGE_2 levels. The effect of respiratory acidosis on PGE_2 degradation has not been determined. Further studies will be necessary to determine whether the differences in prostaglandin levels between metabolic and respiratory acidosis observed in this study are due to differences in production or degradation.

Prostaglandin synthesis is regulated by the release of arachidonic acid from membrane phospholipids through an increase in phospholipase A_2 activity. The subsequent conversion of arachidonic acid to specific prostanoids is catalyzed by prostaglandin G/H synthase (PGHS), also called cyclooxygenase (68). There are two forms of PGHS, both of which are expressed in osteoblasts (59). PGHS-1 is constitutively expressed, and PGHS-2 is the inducible form of the enzyme (68). PGHS-2 expression is regulated by several bone-resorbing factors, including interleukin-1 (44,53,60), parathyroid hormone (43,44,80), interleukin-6 (74), TGF-β (60), TNF-α (43), and basic fibroblast growth factor (42). We have not measured the effect of either metabolic or respiratory acidosis on cellular PGHS-2 RNA or protein levels in calvariae. Determination of the effect of metabolic and respiratory acidosis on PGHS-2 levels will help answer the question of whether the differential effects of these two types of acidosis on PGE_2 levels are due to differences in production or degradation. We have found that another immediate early-response gene, *egr-1*, was stimulated by metabolic acidosis (38), suggesting that PGHS-2 RNA may also increase with metabolic perturbation.

We do not yet know which intracellular signal transduction pathway(s) is activated during the acid-induced increase in prostaglandin synthesis nor what specific cellular receptor is activated in response to acidosis. Although cAMP appears to be the primary second messenger mediating PGE_2-stimulated bone resorption (64), there is evidence for mobilization of intracellular calcium and activation of protein kinase C in osteoblasts (81, 84). In view of the difference in PGE_2 levels between metabolic and respiratory acidosis, the cell must respond to a decrement in HCO_3^- as well as pH. Indeed, we have previously shown that at an equivalent decrement in pH net calcium flux is correlated inversely with medium [HCO_3^-] (27).

This study confirms our previous observations that there is a greater net calcium efflux from bone during metabolic, compared with respiratory, acidosis (7, 11, 22, 28, 70). We had previously questioned whether the greater net calcium efflux from bone during metabolic, compared with respiratory, acidosis was due to greater efflux from or a lesser influx (28) of calcium into bone. Calvariae were prelabeled with ^{45}Ca 24 h before the animals were killed, and medium total and radioactive calcium were determined after cultures simulating metabolic and respiratory acidosis. We found that in live bone there was pH-dependent unidirectional ^{45}Ca release and net calcium efflux from cultured bone in vitro; however, metabolic acidosis produced far greater ^{45}Ca release and net calcium efflux than a comparable degree of respiratory acidosis. Only at a significantly lower pH did the unidirectional ^{45}Ca release and net calcium efflux from bone during respiratory acidosis approach those observed during modest metabolic acidosis. There was a comparable decrease in both unidirectional and bidirectional calcium efflux when bones were incubated in medium with an increased P_{CO_2} compared with an equivalent decrement in pH induced by a decrease in medium HCO_3^-. However, when osteoclasts were inhibited with calcitonin, there was a slightly, but significantly, greater unidirectional calcium efflux than net calcium flux, suggesting that, in addition to less absolute calcium efflux during respiratory acidosis, there also appears to be deposition of medium calcium on the bone during hypercapnia. The present study also confirms previous observations that metabolic acidosis leads to a marked increase in medium PGE_2 levels (46, 61). The magnitude of the increase in medium PGE_2 levels induced by acidosis was similar in this study to those found in our previous report (46).

In the present study, the initial medium pH of the Met and Resp groups were not different during any of the time periods; however, by the end of each incubation the final medium pH was lower in Met than in Resp. We have previously shown that the lowered final pH with Met, compared with Resp, only occurs in incubations in which there are functioning osteoclasts (28). Incubation of calvariae with the osteoclastic inhibitor calcitonin abolishes the difference in final medium pH between incubations in the presence of a low HCO_3^- compared with incubation in the presence of an elevated P_{CO_2}. Because metabolic, but not respiratory, acidosis stimulates osteoclastic function (11), the lower final medium pH in this experiment may be a result of acidosis-induced increased osteoclastic metabolic activity in Met compared with Resp.

Neonatal mouse calvariae in culture respond to protons and calcium-regulating hormones, synthesize DNA and protein, and have functioning osteoblasts and osteoclasts, as human bone does in vivo (73). However, there are differences between cultured mouse

calvariae and human bone. Calvariae are neonatal woven bone compared with mostly mature cortical bone in humans, and in culture calvariae are not perfused by blood. Thus we must exercise caution in applying these in vitro findings to human bone perfused by blood.

Metabolic acidosis induces net calcium efflux from neonatal mouse calvariae initially through physicochemical dissolution and subsequently through cell-mediated mechanisms, consisting of an increase in osteoclastic and a decrease in osteoblastic activity, whereas respiratory acidosis causes neither appreciable calcium efflux nor an alteration in bone cell activity. The present study strongly suggests that this difference in acid-induced, cell-mediated net calcium release is due to an increase in PGE_2 levels with metabolic, but not respiratory, acidosis. Why acidosis produced by a decrement in $[HCO_3^-]$ stimulates PGE_2 production leading to bone calcium efflux, whereas a similar degree of acidosis produced by an increase in P_{CO_2} does not, remains to be determined.

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