Acute metabolic acidosis inhibits the induction of osteoblastic *egr*-1 and type 1 collagen

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Frick, Kevin K., Li Jiang, and David A. Bushinsky. Acute metabolic acidosis inhibits the induction of osteoblastic *egr*-1 and type 1 collagen. *Am. J. Physiol.* 272 (*Cell Physiol.* 41): C1450–C1456, 1997.—Metabolic acidosis induces net calcium efflux from bone through a decrease in osteoblastic formation and an increase in osteoclastic resorption. We tested the hypothesis that changes in external pH would alter the expression of genes critical to the function of mouse calvarial bone cells, predominantly osteoblasts. Cells were cultured in physiologically neutral pH medium until confluent and then stimulated with fresh medium at either neutral or acidic pH. Among a group of immediate early response genes, including *egr*-1, *jun*B, c-*jun*, *jun*D, and c-*fos*, only *egr*-1 stimulation was modulated by changes in medium pH. At pH 7.4, RNA for *egr*-1 was stimulated ~10- to 30-fold, 40 min after medium change. A progressive decrease in pH to 6.8 led to a parallel reduction in *egr*-1 stimulation, and an increase in pH to 7.6 led to an increase in *egr*-1 stimulation. The protein synthesis inhibitor cycloheximide led to a superinduction of *egr*-1 with preservation of the pH dependency of expression. Osteoblasts synthesize collagen, which is subsequently mineralized. RNA for type 1 collagen was stimulated approximately three- to fivefold, 40 min after medium change. Again the stimulation was inhibited by acidosis and increased by alkalosis. Cycloheximide abolished the pH dependency of expression. These results suggest that small changes in external pH have a significant effect on the expression of certain genes important for osteoblastic function.

bone; calcium; gene expression; mineralization

THE PRODUCTION OF METABOLIC acids in mammals is a consequence of endogenous metabolism and is a function of the amount of animal protein in the diet (3). These acids are buffered by extracellular fluid HCO_3^-, causing a decrease in the concentration of serum HCO_3^- that, at a constant partial pressure of CO_2, results in a fall in systemic pH (3). Other body buffers, including the mineral phases of bone, help to mitigate this fall in pH (2). These metabolic acids must ultimately be excreted by renal mechanisms to maintain acid-base homeostasis (3). The clinical disorder of metabolic acidosis may result from impaired excretion of these endogenously produced acids, and the severity of metabolic acidosis is a consequence of both the magnitude of acid production and the efficiency of acid excretion (3). During clinical metabolic acidosis, there is increased urinary calcium excretion without a concomitant increase in intestinal calcium absorption, leading to a net negative calcium balance (23). For maintenance of physiological levels of serum calcium in the face of ongoing urinary losses, calcium must be mobilized from the mineral stores of bone, which contain >98% of total body calcium (35). This calcium release is generally associated with release of bone phosphates and carbonates that contribute to buffering the excess hydrogen ions (2). The high-protein diet of many Americans, which results in the generation of large quantities of metabolic acids, has led some to argue that osteoporosis may result from a lifetime of acid loading, resulting in buffering by bone mineral and calcium release (32).

The effects of metabolic acidosis on bone can be modeled using cultured neonatal mouse calvariae. When calvariae are incubated in medium at a physiologically acidic pH, achieved through a decrease in the medium HCO_3^- concentration at constant partial pressure of CO_2, there is net calcium loss from bone (4, 7, 8). In addition to a component of non-cell-mediated physicochemical bone calcium dissolution (7, 8), metabolic acidosis has been shown to induce cell-mediated calcium efflux from bone (4, 21). Incubation at an acidic pH affects both osteoblastic and osteoclastic function (4, 21). With metabolic acidosis, there is both a decrease in calvarial collagen synthesis and diminished alkaline phosphatase activity, indicating a suppression of osteoblastic function (4, 21). In contrast, the activity of β-glucuronidase, an osteoclastic enzyme, is enhanced by metabolic acidosis (4, 21). These changes indicate an alteration in bone remodeling toward increased resorption and decreased formation, findings consistent with the observed net calcium efflux from bone.

Primary bone cell cultures derived from 4- to 6-day-old mouse calvariae are largely composed of osteoblasts and osteoblast precursors (34). When maintained in an appropriate medium containing β-glycerophosphate and ascorbate, these cultures form regions of mineralization termed bone nodules (15). However, when cultured under conditions simulating metabolic acidosis, there is a marked decrease in the number and calcium content of the bone nodules formed (33). These results indicate that these cultures provide a suitable system to study the effects of acidosis on bone cell function and development.

In this study, we tested the hypothesis that changes in external medium pH would alter the expression of genes critical to the function of osteoblasts. We studied the effects of alterations of pH, within the physiological range, on expression of a group of immediate early response genes, including c-*fos*, the *jun* family, and *egr*-1. We found that metabolic acidosis caused a pronounced, pH-dependent suppression of *egr*-1 expression while having little effect on the expression of the other immediate early response genes studied. A similar effect was observed on the expression of type 1 collagen, an important component of the matrix elaborated by osteoblasts. With both *egr*-1 and type 1 collagen, metabolic alkalosis increased gene expression. These results suggest that modulation of external pH

has a substantial effect on the expression of certain genes important for osteoblastic function.

METHODS

Cell culture. Bone cells were obtained from the calvariae of 4- to 6-day-old Swiss Cox mice. Mice were killed by cervical dislocation, and the calvariae (frontal and parietal bones of the skull) were dissected immediately and then removed to chilled *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES). After accumulation of an appropriate number of bones (20–50), the calvariae were washed in saline-EDTA and then subjected to collagenase (Wako Pure Chemicals, Dallas, TX) digestion. The cells released by collagenase were plated on Primaria plates (Becton Dickinson, Lincoln Park, NJ) in Dulbecco's modified Eagle's medium with 15% heat-inactivated horse serum at a density of 5×10^5 cells/100-mm dish and incubated at 37°C in a CO_2 incubator at a P_{CO_2} of 40 mmHg.

To closely replicate physiological conditions, we used only the HCO_3^-/CO_2 buffer system to control pH after the bone cells were isolated. All medium was preincubated for at least 3 h to assure that the incubator and medium P_{CO_2} reached equilibrium (6). Cells were cultured in neutral pH medium (initial measured means ± SE of all experiments: pH = 7.50 ± 0.07, P_{CO_2} = 39.9 ± 5.8 mmHg, and $[HCO_3^-]$ = 31.0 ± 0.3 meq/l). After 8–10 days, with medium changes every 3–4 days, the cells reached confluence. Medium was changed once more, postconfluence, and the cells were allowed to stabilize for 3–4 days. Some cells in each group were then either *1*) stimulated with fresh neutral pH medium or *2*) stimulated with medium acidified by the addition of concentrated HCl to lower the $[HCO_3^-]$ (initial measured means ± SE of all experiments: pH = 7.12 ± 0.03, P_{CO_2} = 40.4 ± 2.0 mmHg, and $[HCO_3^-]$ = 13.2 ± 0.4 meq/l), or *3*) not stimulated. In additional experiments, the pH was altered in 0.2-pH unit increments by addition of 100 μl of either HCl or $NaHCO_3$, at various concentrations, to 50 ml of neutral medium at P_{CO_2} = 40 mmHg, to lower or increase, respectively, the $[HCO_3^-]$. In other experiments, to test the effect of protein synthesis inhibition on gene expression, cycloheximide (10 μg/ml) was added to confluent cultures 2 h before stimulation and was also included in the stimulating medium. Cells were then harvested, and their RNA was extracted. P_{CO_2} and pH (ABL30 Acid-Base Analyzer, Radiometer, Copenhagen, Denmark) were measured before stimulation and immediately before RNA harvest.

Gene probes and labeling. Probes used for analysis of RNA include c-*fos*, mouse, genomic [American Type Culture Collection (ATCC)] (14); c-*jun*, mouse, cDNA (ATCC) (31); *jun*B, mouse, cDNA (ATCC) (31); *jun*D, mouse, cDNA (ATCC) (30); *egr*-1, mouse, cDNA (ATCC) (11); α_1(1) procollagen (type 1 collagen), rat, cDNA (generous gift of Barbara Kream, University of Connecticut) (18); and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), mouse, cDNA (16). In each case, the inserts were removed from the vector by digestion with the appropriate restriction enzyme(s) and separated by electrophoresis on low-melting-point agarose. Ethidium bromide-stained fragments were identified by ultraviolet (UV) transillumination and excised with a razor blade, and DNA was purified from the gel with Wizard PCR Preps (Promega, Madison, WI). Radioactive probes were prepared by random primer extension, using the Decaprime kit (Ambion, Austin, TX) and [α-^{32}P]deoxycytidine 5′-triphosphate (New England Nuclear, Boston, MA). Unincorporated nucleotides were removed by use of CentriSep spin columns (Princeton Separations, Adelphia, NJ).

RNA extraction and analysis. The cells were quickly scraped into a chaotropic solution (TRI-LS, MRC, Cincinnati, OH) that terminates any stimulation and dissociates RNA from protein complexes. RNA was purified from TRI-LS according to manufacturer's modification of the Chomczynski and Sacchi (10) protocol. After ethanol precipitation, the RNA was dissolved in sterile water at a concentration of 10 μg/μl. Aliquots (15–30 μg) were denatured in 50% formamide-6% formaldehyde by heating to 65°C for 15 min and then electrophoresed on 1% agarose in 3-(*N*-morpholino)propanesulfonic acid (MOPS)-formaldehyde buffer. Samples were routinely stained with ethidium bromide during electrophoresis to ensure the integrity of the rRNA bands. After electrophoresis, the RNA was transferred to a charged nylon membrane (Zeta Probe, Bio-Rad, Richmond, CA) by capillary blotting with 10× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate). After blotting, the nucleic acid was fixed to the membrane by UV cross-linking (Stratalinker, Stratagene, La Jolla, CA). Filters were hybridized and washed according to manufacturer's suggestions; prehybridization (at least 1 h) and hybridization (18–22 h) were conducted in 250 mM sodium phosphate (pH 7.2), 7% sodium dodecyl sulfate (SDS), and 1 mM EDTA at 65°C. After hybridization, the spent solution was removed and the filter(s) was washed twice in 40 mM sodium phosphate (pH 7.2), 5% SDS, and 1 mM EDTA at 65°C and then twice in 40 mM sodium phosphate (pH 7.2), 1% SDS, and 1 mM EDTA at 65°C. For filters probed for type 1 collagen, two additional washes with 0.1× SSC-0.5% SDS at 65°C were included. The washed filters were autoradiographed on Kodak XAR-5 film, and the signal was quantified by use of a Molecular Dynamics PhosphorImager. To normalize for possible variations in gel loading, we routinely stripped filters of probe by two 20-min washes in 0.1× SSC-0.5% SDS heated to 100°C; the stripped filters were reprobed with GAPDH, and the signal was quantitated as above.

Statistical analysis. Tests of significance were calculated by analysis of variance and linear regression analysis, using conventional computer programs (BMDP Statistical Software, Los Angeles, CA). All values are presented as means ± SE; $P < 0.05$ was considered significant.

RESULTS

When the cells were not stimulated by a medium change, only trace amounts of c-*jun* RNA were observed (Fig. 1*A*). However, when the cells were stimulated by medium at physiologically neutral pH, there was a marked increase in the expression of c-*jun* RNA, with maximum levels observed at 40–60 min. Stimulation with physiologically acidic medium resulted in a similar pattern of c-*jun* expression. Similarly, there were only trace amounts of *jun*B RNA in nonstimulated cells, which increased rapidly after medium change, reaching maximum levels at 30 min; acidic medium did not alter *jun*B stimulation. In contrast, detectable quantities of *jun*D RNA were observed in nonstimulated cells; after medium change, there was no increase in *jun*D RNA levels until ~90 min. This pattern of *jun*D stimulation was also not altered by acidic medium.

The genomic c-*fos* probe hybridized strongly to two regions near the appropriate size in RNA from nonstimulated cells (Fig. 1*B*; respective GAPDH, Fig. 1*D*). Despite the substantial basal level, c-*fos* RNA increased in abundance quickly after medium change. At neutral pH, maximal c-*fos* RNA expression was observed at

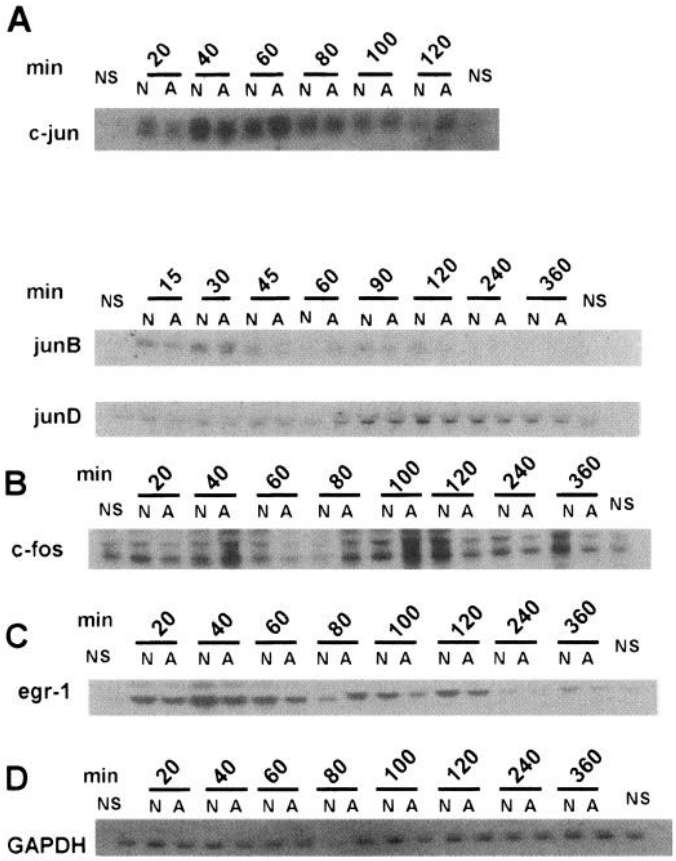

Fig. 1. Effects of acid incubation on immediate early gene expression. Cultured neonatal mouse calvarial bone cells were stimulated by neutral or acidic medium change or left nonstimulated and then harvested at indicated times for preparation of RNA. NS, cells not stimulated by medium change; N, cells stimulated with neutral medium (pH = 7.50 ± 0.07, P_{CO_2} = 39.9 ± 5.8 mmHg, and $[HCO_3^-]$ = 31.0 ± 0.3 meq/l); A, cells stimulated with acidic medium (pH = 7.12 ± 0.03, P_{CO_2} = 40.4 ± 2.0 mmHg, and $[HCO_3^-]$ = 13.2 ± 0.4 meq/l); time (min) represents time from beginning of stimulation. For autoradiographs, 20 μg total RNA/lane were electrophoresed in 1% agarose containing formaldehyde and then transferred to a nylon membrane via Northern blotting. *A*: 3 separate filters were probed for c-*jun*, *jun*B, and *jun*D. *B–D*: 1 filter was probed for c-*fos*, *egr*-1, and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), respectively.

15–20 min. In contrast, the expression of c-*fos* RNA in cells stimulated by acid medium appeared to lag behind control levels and to reach a peak level at 30–40 min poststimulation. There was no difference in the maximal levels of c-*fos* RNA between cells stimulated in acidic or neutral pH medium.

Stimulation of *egr*-1 RNA was rapid and pronounced in cells stimulated by neutral medium (Fig. 1*C*; respective GAPDH, Fig. 1*D*). At 40 min, the amount of *egr*-1 RNA increased ~10- to 30-fold over basal levels. Compared with neutral medium, there was 30–50% less stimulation (n = 5 experiments) with acidic medium (Fig. 2). The time course of *egr*-1 stimulation did not differ between cells exposed to neutral or acidic medium.

To determine whether the degree of *egr*-1 RNA stimulation was pH dependent, we varied the pH of the stimulating medium between 6.8 and 7.6 (in 0.2-unit increments) at P_{CO_2} of 40 mmHg. RNA was harvested

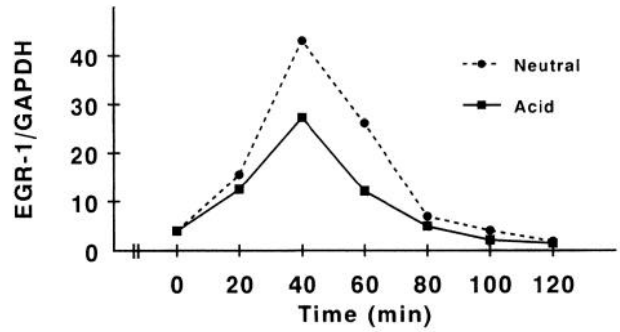

Fig. 2. Time course of *egr*-1 stimulation. After Northern blotting and probing for *egr*-1, filter was quantitated using a Molecular Dynamics PhosphorImager. For normalized values, filter was then stripped of hybridized probe, rehybridized with a probe for GAPDH, and quantitated as above. All values indicated are ratio of *egr*-1 to GAPDH. Data are from a typical experiment, different from that illustrated in Fig. 1, *C* and *D*.

at a time point previously determined to be at peak *egr*-1 expression (40 min). The *egr*-1 RNA expression was pH dependent; a progressive decrease in medium pH from 7.4 to 6.8 led to a parallel reduction in *egr*-1 stimulation, and an increase in pH to 7.6 led to an increase in stimulation (Fig. 3*A*, autoradiogram from a

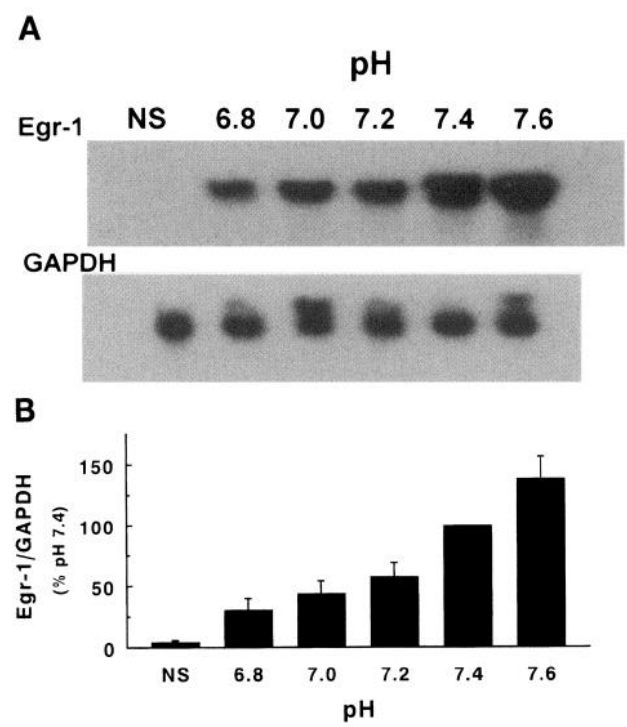

Fig. 3. Response of *egr*-1 RNA to stimulation with medium of various pH. Cultured neonatal mouse calvarial cells were stimulated for 40 min with medium at indicated pH and then harvested for RNA preparation; as a further control, some cells were not stimulated (NS). Medium pH was altered by modification of $[HCO_3^-]$ at a constant P_{CO_2} of 40 mmHg. *A*: autoradiographs of a typical Northern filter, as probed for *egr*-1 (*top*) and then reprobed for GAPDH (*bottom*); 20 μg total RNA were electrophoresed in each lane. *B*: means ± SE from 5 repetitions, graphed as %stimulation at pH 7.4 (all values corrected for GAPDH RNA content). NS differed from all stimulated samples; pH 6.8 differed from pH 7.2, 7.4, and 7.6; pH 7.0 differed from pH 7.4 and 7.6; pH 7.2 differed from pH 7.4 and 7.6; and pH 7.4 differed from pH 7.6 ($P < 0.05$ in all cases).

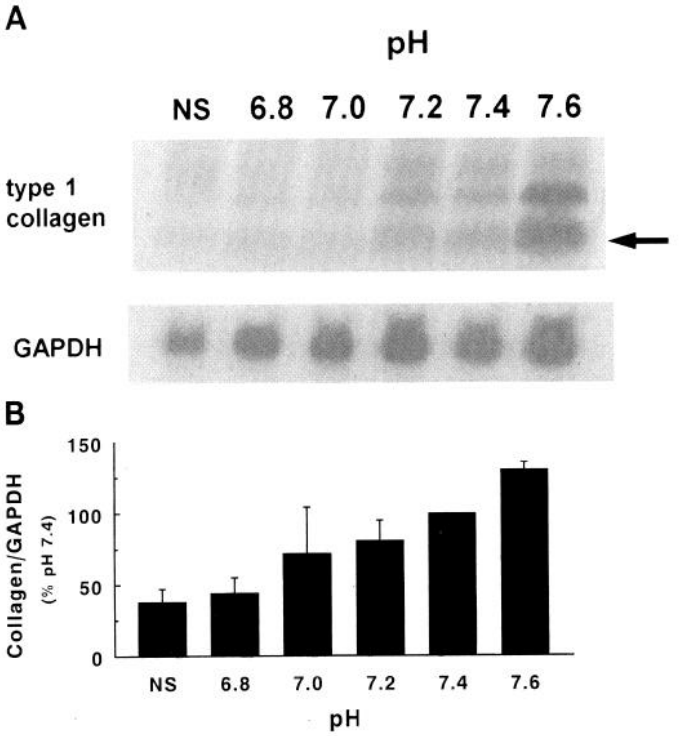

Fig. 4. Response of type 1 collagen RNA to stimulation with medium of varying pH. Cultured neonatal mouse calvarial cells were stimulated for 40 min with medium at indicated pH and then harvested for RNA preparation; 20 μg total RNA were electrophoresed in each lane. As a further control, some cells were not stimulated (NS). Medium pH was altered by modification of $[HCO_3^-]$ at a constant P_{CO_2} of 40 mmHg. A different filter was used than for Fig. 3. *A*: autoradiographs of a typical filter, as probed for type 1 collagen (*top*) and reprobed for GAPDH (*bottom*). *B*: means ± SE from 3 repetitions, graphed as %stimulation at pH 7.4 (all values corrected for GAPDH RNA content). Because of multiple bands of hybridization with type 1 collagen probe, lowest band (*A*, arrow) was used for quantitation. Similar results were obtained when doublet immediately above lowest band was used for quantitation. NS differed from pH 7.4 and 7.6; pH 6.8 differed from pH 7.4 and 7.6; and pH 7.4 differed from pH 7.6 ($P < 0.05$ in all cases).

representative experiment). The levels of *egr*-1 RNA obtained from cells stimulated with medium at pH 7.4 were significantly different from the levels seen at pH 6.8, 7.0, or 7.2 or at pH 7.6 ($n = 5$, $P < 0.05$ for each comparison; Fig. 3*B*). There was a significant direct correlation between *egr*-1 expression and pH ($r = 0.913$, $n = 25$, $P < 0.001$).

To test the possibility that a gene known to be important in osteoblast function was also affected by the pH of the stimulation medium, we examined RNA for type 1 collagen. RNA for type 1 collagen was stimulated approximately three- to fivefold by neutral pH medium, 40 min after the medium change (Fig. 4*A*, autoradiogram from a representative experiment). Hybridization of type 1 collagen revealed the presence of a distinct lower band and a more slowly migrating doublet; similar results were obtained by quantitation of either the lower band or the doublet. As with *egr*-1, the induction was inhibited in a graded manner by decreasing the medium pH to 6.8 and was stimulated by increasing the medium pH to 7.6 (Fig. 4*B*). There was a direct correlation between collagen stimulation and medium pH ($r = 0.805$, $n = 15$, $P < 0.001$).

To determine whether de novo protein synthesis was required for the dependence of gene expression on pH, we utilized the protein synthesis inhibitor cycloheximide. Cells were preincubated for 2 h with cycloheximide, to ensure adequate levels of inhibition, and then stimulated with fresh medium in the presence of cycloheximide. In the absence of stimulation, cycloheximide caused a pronounced increase in *egr*-1 RNA (Fig. 5*A*). After stimulation with fresh medium at pH 7.4, there was a superinduction of *egr*-1 RNA with cycloheximide (Fig. 5*B*). In the presence of the superinduction, there remained a pH dependence on the degree of *egr*-1 expression. Stimulation at pH 6.8 and 7.0 induced less *egr*-1 RNA than did stimulation at the physiologically neutral pH of 7.4. There was no increase in *egr*-1 RNA at pH 7.6 compared with pH 7.4.

In contrast to *egr*-1, type 1 collagen RNA was not stimulated by cycloheximide alone (Fig. 5*A*). Medium change in the presence of cycloheximide resulted in an increase in type 1 collagen RNA levels; however, there was no superinduction of the RNA with cycloheximide (Fig. 5*C*). Cycloheximide abolished the pH dependence of type 1 collagen RNA.

DISCUSSION

Metabolic acidosis has been shown to decrease some osteoblastic functions, including collagen synthesis, and this decrease is thought to be, in part, responsible for the acidosis-induced increase in net calcium efflux from bone. In this study, we tested the hypothesis that a decrease in medium pH would alter expression of genes critical to the function of osteoblasts. We examined the expression of certain immediate early genes and of type 1 collagen in medium-stimulated bone cells. The expression of the immediate early response genes c-*fos*, c-*jun*, *jun*B, and *jun*D was not appreciably altered by differences in pH within the physiological range. However, induction of both *egr*-1 and type 1 collagen was inhibited by acidic medium and stimulated by alkaline medium.

At its peak stimulation, which occurred at 40 min, *egr*-1 was increased some 10- to 30-fold by the medium change. The time to peak stimulation did not differ between the cells stimulated by neutral or acidic medium; however, the peak expression in cells stimulated at physiologically neutral medium was two- to threefold greater than in acid-stimulated cells. This pH dependency of *egr*-1 response was further examined by progressive adjustment of the pH of the stimulating medium. Decreasing the external pH by as little as 0.2 units caused a significant decrease in *egr*-1 stimulation, compared with the degree of stimulation measured at neutral pH medium. In contrast, increasing the pH of the stimulating medium, again by 0.2 units, increased *egr*-1 expression. The degree of *egr*-1 induction was correlated directly to the pH of the stimulating medium ($r = 0.913$, $n = 25$, $P < 0.001$).

RNA for type 1 collagen is prevalent in these cells before stimulation by medium change. Stimulation by

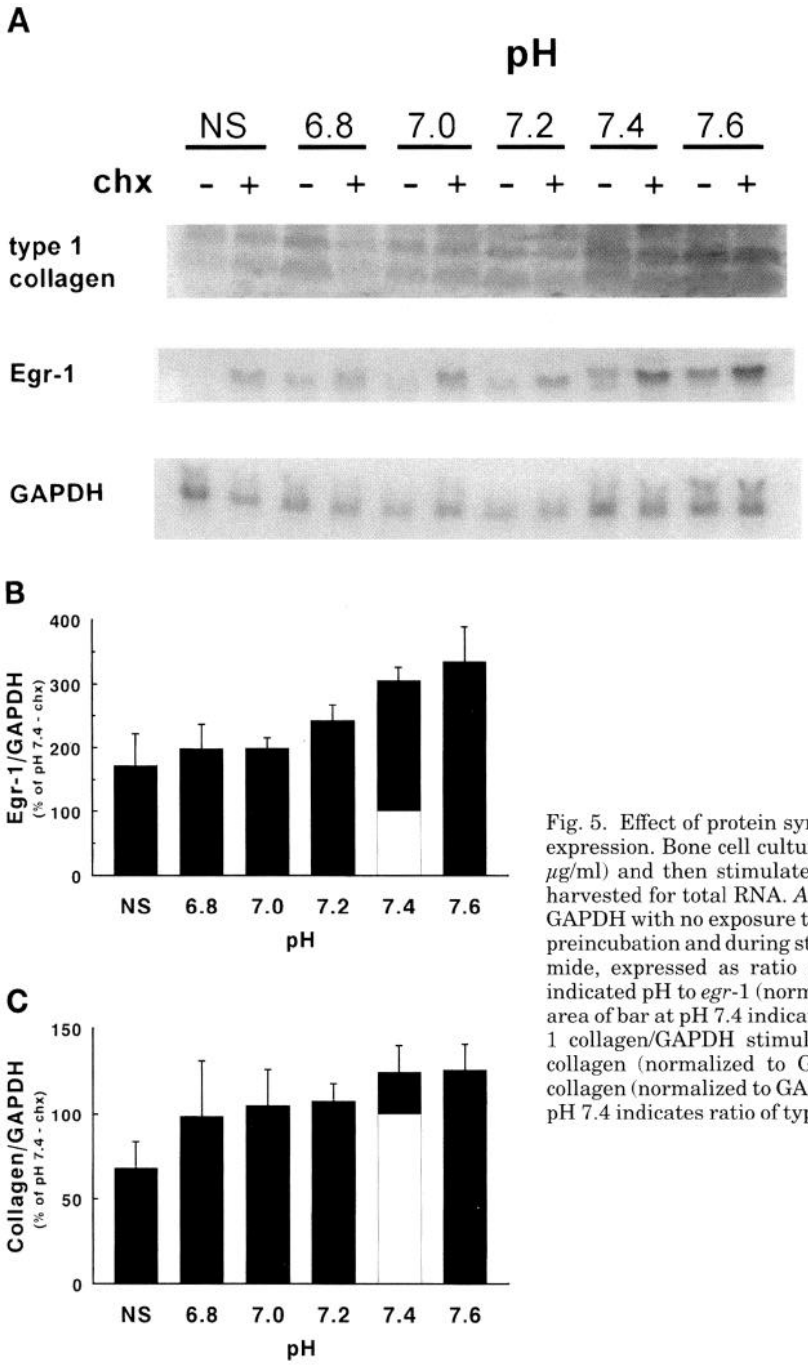

Fig. 5. Effect of protein synthesis inhibition on pH dependence of *egr*-1 and collagen expression. Bone cell cultures were preincubated for 2 h with cycloheximide (chx; 10 μg/ml) and then stimulated at indicated pH for 40 min, at which point cells were harvested for total RNA. *A*: Northern blot showing expression of *egr*-1, collagen, and GAPDH with no exposure to cycloheximide (−) or with cycloheximide present both for preincubation and during stimulation (+). *B*: *egr*-1/GAPDH stimulation with cycloheximide, expressed as ratio of *egr*-1 (normalized to GAPDH) with cycloheximide at indicated pH to *egr*-1 (normalized to GAPDH) without cycloheximide at pH 7.4. Open area of bar at pH 7.4 indicates ratio of *egr*-1 to GAPDH without cycloheximide. *C*: type 1 collagen/GAPDH stimulation with cycloheximide, expressed as ratio of type 1 collagen (normalized to GAPDH) with cycloheximide at indicated pH to type 1 collagen (normalized to GAPDH) without cycloheximide at pH 7.4. Open area of bar at pH 7.4 indicates ratio of type 1 collagen to GAPDH without cycloheximide.

neutral medium resulted in a three- to fivefold increase in type 1 collagen RNA. As observed with *egr*-1, this stimulation was reduced by a decrease in pH and augmented by an increase in pH. There was a direct correlation of type 1 collagen stimulation with medium pH ($r = 0.805$, $n = 15$, $P < 0.001$).

The presence of consistent levels of GAPDH RNA throughout the pH range examined in this study indicates that the differences observed with *egr*-1 and type 1 collagen RNA expression are not a result of overt cytotoxicity but rather a reflection of the response to a change in medium pH. When the mean results for *egr*-1 and type 1 collagen for each pH were compared, they were directly correlated ($r = 0.968$, $n = 5$, $P = 0.007$). This robust correlation only suggests that pH has a uniform effect on osteoblastic expression of some genes and does not necessarily imply that there is any interrelationship between the two genes.

Inhibition of protein synthesis with cycloheximide has differing effects on *egr*-1 and type 1 collagen RNA levels. Cycloheximide alone caused an increase in *egr*-1 RNA, as would be expected for an unstable mRNA species (17). Stimulation with fresh medium caused an increase in *egr*-1 RNA abundance even in the presence of cycloheximide, with the degree of stimulation remaining pH dependent. In contrast, cycloheximide alone did not cause an increase in type 1 collagen RNA levels, and cycloheximide treatment abolished the pH dependence

of type 1 collagen stimulation. It is unclear from this study whether the changes in *egr*-1 affect type 1 collagen directly, as would be expected if EGR-1 acts as a transcription factor on the collagen gene. Further studies, including direct measurement of transcription and DNA-protein interactions, will be required to understand any interrelationship between *egr*-1 and type 1 collagen.

Yamaji et al. (36) showed that *egr*-1 stimulation was dependent on pH in a renal proximal tubule cell line. However, in contrast to our findings of acid medium suppressing *egr*-1 stimulation, in their system there was a greater induction of *egr*-1 with acidic than with neutral pH medium. This clear difference in the directional dependency of *egr*-1 stimulation on pH may stem from the differing roles of these two cell types. A primary function of renal tubule cells is to excrete acid into the glomerular ultrafiltrate. In renal proximal tubular cells, acidosis is known to stimulate renal ammonia synthesis and proton transport, both of which are necessary for increased acid excretion (1). However, in response to metabolic acidosis there is net resorption of bone mineral due, in part, to a decrease in osteoblastic bone formation (4, 21). Thus an increase in *egr*-1 stimulation in renal cells and a decrease in osteoblasts is consistent with their differing functions in response to metabolic acidosis.

Expression of *egr*-1 appears to be modulated in response to a changing environment. Osteoblasts express *egr*-1 in response to H_2O_2 (25) or increased gravitational force (24). In renal epithelial cells, calcium oxalate crystals increase *egr*-1 expression (19), as do urea (12) and hyperosmotic stress (13). Hemodynamic stress increases *egr*-1 expression in myocardial cells (29). Although EGR-1 protein binds selectively to a guanine- and cytosine-rich DNA sequence and could presumably regulate transcription (17), the effects of alterations in *egr*-1 induction in this system are not clear. The human tumor necrosis factor-α (TNF-α) gene promoter is reported to contain an *egr*-1-responsive site (20), and TNF is known to induce bone resorption (28). Mice with a null mutation in the *egr*-1 gene have no obvious altered phenotype, suggesting that the role of *egr*-1 may be replaceable in vivo (22).

In contrast to *egr*-1, there was no difference in the stimulation between acid and neutral medium with respect to the *jun* family members, including c-*jun*, *jun*B, and *jun*D. There was some difference in the time course, but not the magnitude, of c-*fos* stimulation between acid and neutral medium. Because c-Fos/Jun dimers [activator protein 1 (AP-1)] (9) are known to regulate transcription of multiple genes, the apparent alteration in this ratio might affect osteoblastic transcription (27). Further studies will be necessary to determine whether there are alterations in AP-1 activity in this system and, if so, whether they are of physiological significance.

The mechanism(s) by which osteoblastic cells detect changes in medium pH was not addressed in this study. Changes in external pH lead to rapid changes in intracellular pH (26), so it is possible that the effects of acid conditions are direct, altering, for example, the affinity of particular transcription factors toward their binding sites or the ability of protein kinases to activate downstream targets. Further studies will be necessary to determine how *egr*-1 and type 1 collagen synthesis are coupled to changes in pH.

Metabolic acidosis increases bone resorption through an increase in osteoclastic function and a decrease in osteoblastic function. In bone organ culture, we have shown that osteoblastic collagen synthesis is decreased during metabolic acidosis (4, 21) and increased during metabolic alkalosis (5). The results of this study, in which we show that acidosis decreases type 1 collagen RNA expression, are consistent with our prior observations. The relationship, if any, between modulation of the expression of early immediate response gene *egr*-1 and the differentiated osteoblastic function of type 1 collagen synthesis must be determined in future studies.

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